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BASAL METABOLISM STANDARDS

A STATISTICAL COMPARISON OF THEIR PREDICTION VALUES

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THE present study was undertaken in an effort to improve the prediction value of basal metabolism standards. This effort was unsuccessful, in that, as will be presently shown, no significant improvement was realized; but in the process a statistical comparison of the prediction value of various standards now in use was made. The purpose of this article is to present the results of this comparison, and to stress certain principles in the comparison of standards overlooked by several previous writers.

Comparisons of predicted basal metabolism with that obtained by experimental measurement have commonly been made by calculating the average deviation of the observed metabolism from the predicted metabolism. This practice has been a most unfortunate one and has contributed confusion rather than clarity to the problem. It violates what appears to the writer as a fundamental principle of basal metabolism standards—that these standards are to be regarded as relative and not absolute. Apparently climate and race influence the basal metabolism. Be the cause what it may, the determination of basal metabolism in various laboratories gives results which frequently parallel each other at different levels. The calculation of *average deviation* does not in any way distinguish between prediction errors which are a result of the failure of the predicted values to *parallel* the observed values, and those which are the result of a difference in the *level* regarded as normal. To take a hypothetical, extreme example, let us assume that we have 100 normal men whose metabolism has been determined and expressed in terms of standards A and B. According to standard A, which was worked out in the laboratory in which these men have been studied, the basal metabolism of these men ranges from minus 12 per cent to plus 12 per cent with an average deviation of let us say 6 per cent. According to

standard B which was perhaps worked out in a somewhat colder climate, the basal metabolism ranges from minus 11 per cent to minus 9 per cent with an average deviation of 10 per cent. By the comparison of average deviations, the former standard would be the better. Actually the latter

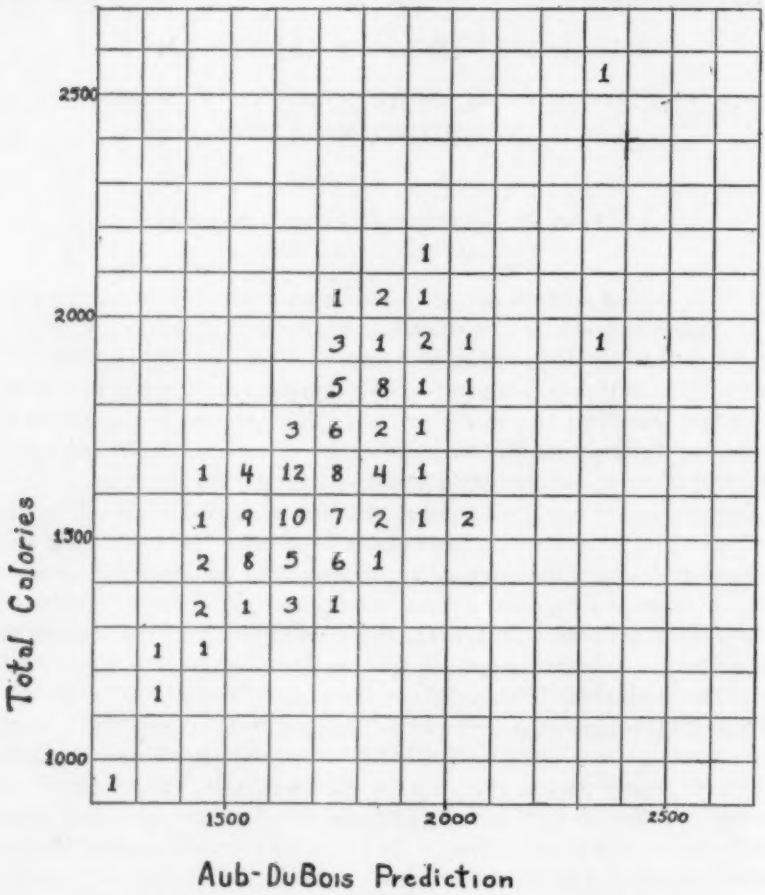

FIG. 1

gives a much more accurate relative prediction, and if 10 per cent were added to each determination, it would give a far more accurate absolute prediction. It is not necessary in clinical use to add the 10 per cent, but simply to revise one's view of what is normal metabolism in this locality as determined in this laboratory according to standard B.

This principle may be stated as follows: *the empirical test of a basal metabolism standard is its accuracy in the relative prediction of the basal metabolism of normal human beings.* Its corollary is; *for the clinical applica-*

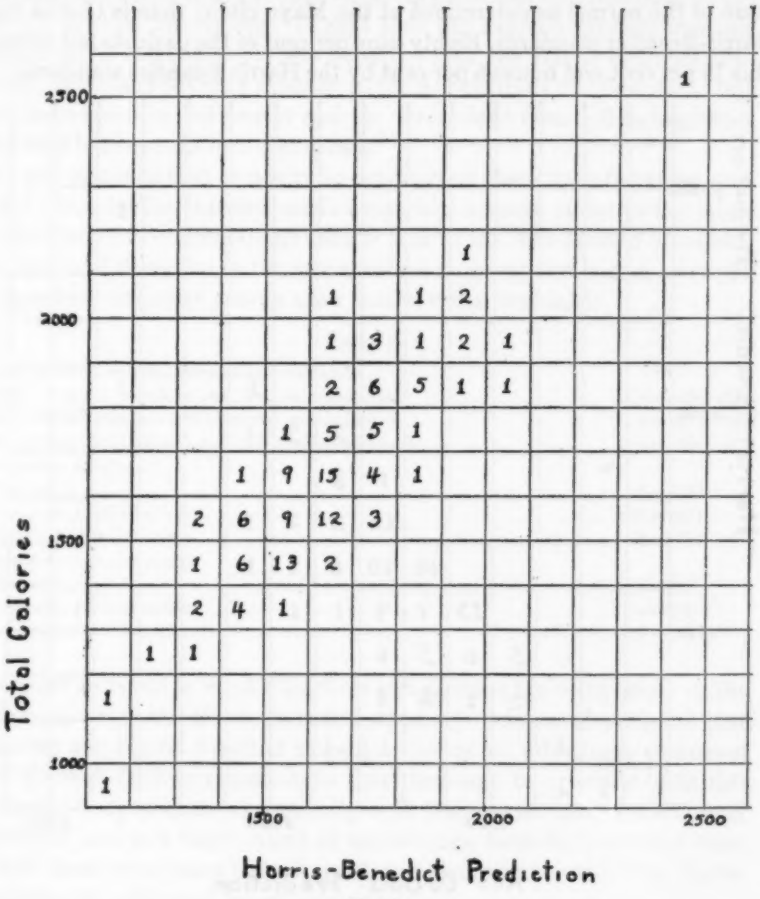

FIG. 2

tion of any basal metabolism standard there should be available the mean basal metabolism of normal individuals of the region, determined under the conditions used for clinical determinations. The above principle was recognized by Stoner (1) in his criticism of Boothby and Sandiford (2). The latter writers compared the metabolism of 127 normal people as determined by the Harris-Benedict and Aub-DuBois standards. They found 92 per cent

of the determinations within ±10 per cent by the Aub-DuBois standard, and only 82 per cent by the Harris-Benedict. As Stoner points out, this is because the *mean* of the Aub-DuBois is more nearly coincident with the *mean* of the normal as determined at the Mayo clinic, than is that of the Harris-Benedict standards. Eighty-nine per cent of the patients fall within plus 14 per cent and minus 6 per cent by the Harris-Benedict standards.

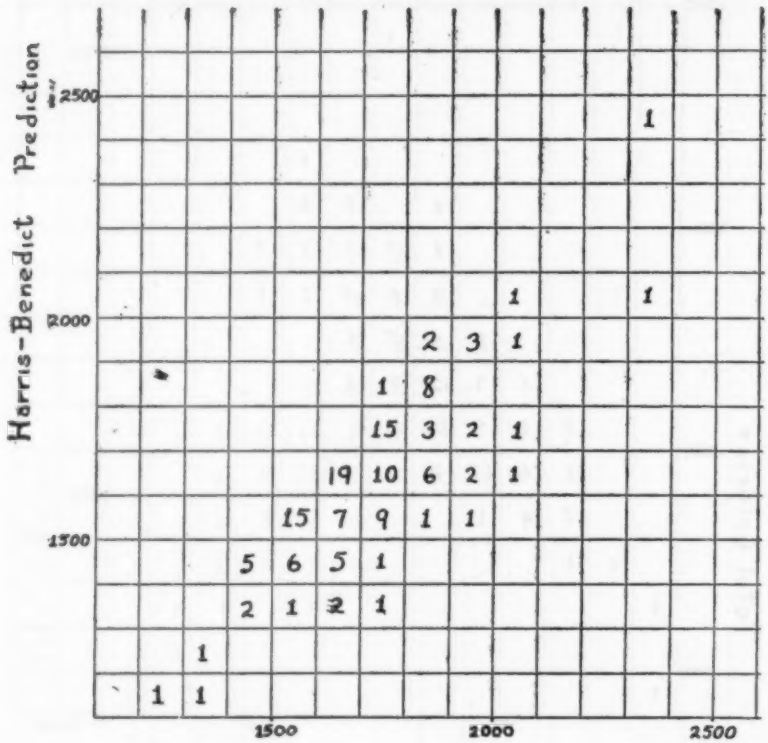

FIG. 3

Two methods are available for the comparison of relative prediction values with facility. One is the comparison of scatter diagrams of predicted caloric production with those obtained by calorimetry, direct or indirect, and the calculation of correlation coefficients. The other is the calculation of the standard deviation of errors of prediction.

Standards for Adult Males

Figures 1, 2 and 3 are scatter diagrams of observed and predicted caloric consumption based upon the data on males published by Harris and Benedict (3) in their monograph. Table I lists the Pearson product-moment coefficients of correlation. It will be noted that the Harris-Benedict prediction formula is not only superior to the Aub-DuBois on these data, but that the correlation between it and the determined metabolism is greater than that between the two standards.

It will be noted that as might be expected on these data the mean predicted value by the Harris-Benedict standard is much closer to the mean of the observed determinations than is that of the Aub-DuBois standard. The standard deviation of the actual caloric consumption is also, as might be expected, distinctly greater than that of either standard.

Table I

Pearson product-moment correlation coefficients	
Harris-Benedict standard and observed metabolism	$r = .84 \pm .031$
Aub-DuBois standard and observed metabolism	$r = .69 \pm .042$
Harris-Benedict standard and Aub-DuBois standard	$r = .80 \pm .035$
Mean value, calories	
Observed	$M = 1635$
Predicted, Harris-Benedict	$M = 1631$
Predicted, Aub-DuBois	$M = 1712$
Standard deviation, calories	
Observed	$\sigma = 210$
Predicted, Harris-Benedict	$\sigma = 176$
Predicted, Aub-Dubois	$\sigma = 168$

It must be borne in mind that these comparisons are being made on the data from which the Harris-Benedict standards were developed, and that therefore the Harris-Benedict standards are at an advantage compared with the Aub-DuBois standards in that they may be expected to fit this particular body of data exceptionally well. This is also true of the Dreyer standards, and to a lesser extent of the two new formulae presented here, in that these were based upon a portion of the cases presented by Harris and Benedict, or in part based upon them.

In order to make a more extensive comparison of standards, the predicted caloric consumption in 24 hours by each of a series of standards was expressed in terms of the observed consumption. This is the reverse of the usual procedure. Inasmuch as this investigation is being made of the *standards* instead of the metabolic rate of particular cases, it is the more logical method here and was also the simpler procedure in mass calculations. The mean and the standard deviation of each series of percentages

were then calculated. Inasmuch as a standard deviation of 5 per cent does not have the same quantitative significance with a mean of 110 per cent as it has with a mean of 95 per cent, it was decided to correct all standard deviations to a mean of 100 per cent by dividing each by the corresponding mean. This gives a figure which, if one drops the per cent sign, is numerically equal to the coefficient of variation.[1] The results are tabulated in Table II.

TABLE II

PREDICTION VALUE OF VARIOUS STANDARDS ON DATA OF HARRIS AND BENEDICT
(Men only, 134 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Aub-DuBois
	Per cent	Per cent	Per cent	
Harris-Benedict	6.10±.250	±4.11	100.4±.36	−5.6
Power formula for adults	6.15±.252	±4.14	99.5±.36	−6.5
General power formula	6.46±.266	±4.36	100.5±.37	−5.4
Dreyer (observed weight)	6.58±.270	±4.44	99.4±.38	−6.5
Mayo	8.28±.341	±5.58	108.2±.52	+2.3
Bailey	8.55±.352	±5.77	106.6±.53	+0.7
Aub-DuBois	8.59±.353	±5.79	105.9±.53	+0.0
Krogh	8.64±.354	±5.83	97.7±.50	−8.3

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different probable error.

From the corrected standard deviation, the corrected probable error was obtained by multiplying by .6745. The formulae and standards are arranged in order of their relative prediction accuracy—inversely as the magnitude of their standard deviations. The Harris-Benedict standard (based upon the Harris-Benedict tables) gives slightly the best relative prediction. The author's power formula for adults ($78.36\ \text{Age}^{-.142}\ \text{Ht.}^{.214}\ \text{Wt.}^{.575}$) runs an insignificant shade behind. The writer's general power formula, for both adults and children ($6.77\ \text{Age}^{-.122}\ \text{Ht.}^{.831}\ \text{Wt.}^{.345}$) comes third, followed by the Dreyer standard based on observed weight (4) (calculations based upon the tables published by Stoner) (5, 6). There is then a significant

[1] Whereas the coefficient of variation has a probable error very complex to calculate, (because account is taken of the error of the mean as well as the error of the standard deviation) the probable error of the standard deviation is very simple to calculate, being $.6745\ \sigma/2N$ and can be corrected by dividing by the mean in the same fashion as the standard deviation itself. The result is a figure spuriously but negligibly lower than the probable error of the coefficient of variation. The corrected standard deviation was used in place of the coefficient of variation because of the simplicity of the calculation of its probable error.

drop in prediction value to the standards based upon the DuBois height-weight formula (7) for surface area. (The surface area was taken from the DuBois table.) Of these, the standard (8) published by Boothby and Sandiford, called here the Mayo standard for convenience, gives slightly the best result, followed by the Bailey standard (9), the original Aub-DuBois (10) and The Krogh standards (11) in order.

A considerable variation is to be noted in the mean of the various standards. The lowest standard (which, of course, would tend to give the high-

TABLE III

PREDICTION VALUE OF VARIOUS STANDARDS ON DATA OF HOBSON
(Males, 46 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Bailey
	Per cent	Per cent	Per cent	
Dreyer (observed weight)	6.10±.429	±4.11	94.9±.58	−5.2
Mayo	6.26±.440	±4.22	101.4±.63	+0.3
Dreyer (calculated weight)	6.50±.458	±4.39	97.5±.63	−2.6
Hobson's modification of Dreyer's formula	6.55±.461	±4.42	100.6±.66	+0.5
Bailey	6.62±.465	±4.47	100.1±.66	0.0
General power formula	6.79±.478	±4.58	93.5±.63	−6.6
Power formula for adults	6.81±.478	±4.59	93.5±.63	−6.6
Harris-Benedict	7.19±.506	±4.85	94.0±.67	−6.1

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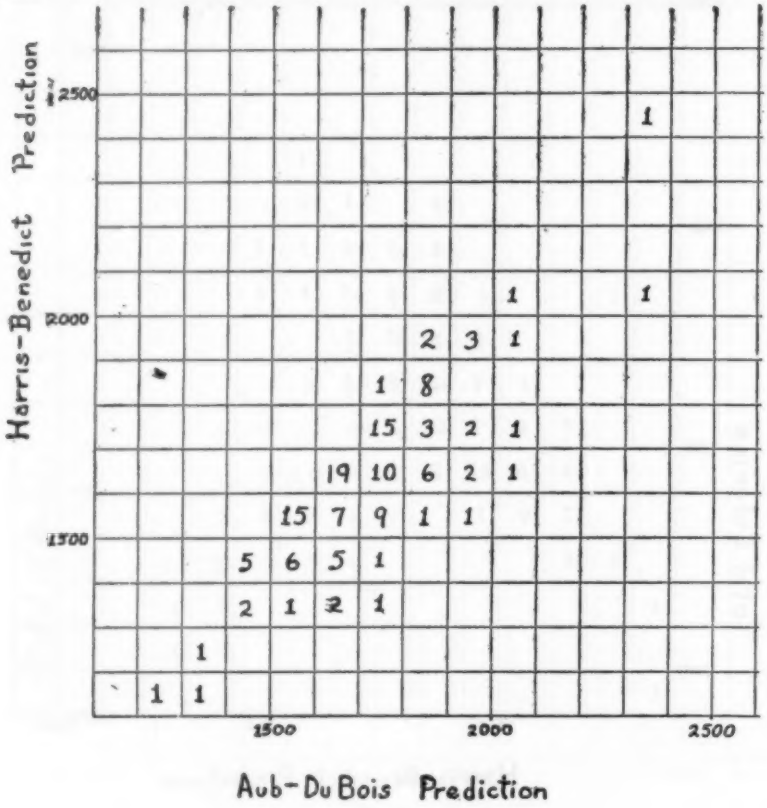

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on observed weight gives the best agreement. The relatively poor agreement given by the standards based upon the body surface law in the data of Harris and Benedict is not apparent here. The mean values are not

TABLE IV

PREDICTION VALUE OF VARIOUS STANDARDS ON DATA OF BOOTHBY AND SANDIFORD
(Men only, 41 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Aub-DuBois
	Per cent	Per cent	Per cent	
Harris-Benedict	5.95±.443	±4.00	94.8±.59	−4.4
Dreyer (observed weight)	5.96±.444	±4.02	94.3±.59	−4.9
Mayo	5.86±.436	±3.95	99.7±.62	+0.4
Krogh	6.14±.457	±4.15	93.1±.60	−6.0
Aub-DuBois	6.19±.462	±4.17	99.2±.65	0.0
Bailey	6.19±.462	±4.17	100.2±.65	+1.0

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different probable error.

TABLE V

PREDICTION VALUE OF VARIOUS STANDARDS ON DATA OF EARLE
(Chinese men, 87 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Aub-DuBois
	Per cent	Per cent	Per cent	
Harris-Benedict	6.59±.337	±4.44	107.6±.51	−2.5
Dreyer (observed weight)	6.85±.351	±4.62	109.8±.54	−0.3
Krogh	7.10±.363	±4.79	105.1±.54	−5.0
Aub-DuBois	7.13±.364	±4.81	110.1±.57	0.0
Bailey	7.20±.368	±4.86	110.9±.58	+0.8
Mayo	7.26±.371	±4.90	113.4±.60	+3.3
Dreyer (calculated weight)	7.70±.394	±5.20	110.0±.61	−0.1

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different probable error.

widely changed with relation to each other, but these cases consumed relatively more oxygen as a group, than did the previous ones. It should be noted that the substitution of calculated weight for observed weight in Dreyer's formula and the modification of the formula made by Hobson to fit these particular cases both actually diminish the relative prediction

value of the formula. No evidence is forthcoming that there is any advantage in either of these modifications, over Dreyer's original formula. Inasmuch as the Harris-Benedict published tables do not cover the age range in this table (although the writers say the formula is applicable) it was necessary here and occasionally later to make calculations directly from the formula.

In Table IV are entered the data obtained by a comparison of the forty-one normal males published by Boothby and Sandiford (13). The general level of calorie consumption is greater in this body of data than in either of the previous ones. This is reflected in the high level of the Mayo standards published by Boothby and Sandiford.

TABLE VI

PREDICTION VALUE OF VARIOUS STANDARDS ON DATA OF AUTHOR
(College freshmen, men only, 34 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Aub-DuBois
	Per cent	Per cent	Per cent	
Harris-Benedict	7.04± .576	±4.75	102.4± .83	−5.6
Dreyer (observed weight)	7.11± .582	±4.79	101.5± .84	−6.5
Krogh	7.84± .643	±5.29	100.9± .92	−7.1
Mayo	7.90± .648	±5.33	109.5±1.00	−1.5
Bailey	8.08± .662	±5.45	107.4±1.01	−0.6
Aub-DuBois	8.27± .677	±5.58	108.0±1.03	0.0

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different probable error.

Further data are treated in Tables V and VI. Table V combines series A, B, and C published by Earle (14). These are all determinations upon Chinese men in Hongkong and seem comparable. It will be noted that again the Dreyer standard based upon calculated weight is inferior to the Dreyer standard based on observed weight. As Boyd (15) has pointed out, calculating body weight from sitting height (and one might certainly add, from chest circumference) means the substitution of a less accurate determination (sitting height or chest circumference) for a more accurate one. Table VI contains data on 34 male university freshmen from determinations by the author.

The Harris-Benedict formula gives on the whole the best relative prediction on the data available on men, with the Dreyer standard (observed weight) and the Mayo standard following.

Standards for Women

The tabulation of data from the material of Harris and Benedict (3) on women is contained in Table VII. Here there are no significant differences in relative prediction accuracy, although the Dreyer (observed weight) prediction is slightly the best on this particular body of data. The differences in mean level roughly parallel those found in the data of these authors on men.

Table VII

Prediction Value of Various Standards on Data of Harris and Benedict
(Women only, 103 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Aub-DuBois
	Per cent	Per cent	Per cent	
Dreyer (observed weight)	7.68±.361	±5.17	99.8±.51	−4.8
Krogh	7.72±.363	±5.20	98.6±.51	−6.1
Mayo	7.78±.366	±5.24	103.3±.53	−1.3
Aub-DuBois	7.82±.367	±5.27	104.7±.54	0.0
Harris-Benedict	7.85±.369	±5.29	100.8±.33	−3.8
Bailey	8.21±.386	±5.54	105.0±.57	+0.4

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different probable error.

Table VIII

Prediction Value of Various Standards on Data Of Boothby and Sandiford
(Women only, 61 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Aub-DuBois
	Per cent	Per cent	Per cent	
Bailey	5.02±.306	±3.39	100.8±.44	+0.5
Krogh	5.46±.333	±3.68	94.2±.44	−6.1
Mayo	5.55±.339	±3.74	99.1±.48	−1.2
Aub-DuBois	5.59±.341	±3.77	100.3±.48	0.0
Harris-Benedict	5.85±.357	±3.95	96.3±.49	−4.0
Dreyer (observed weight)	6.17±.377	±4.16	95.6±.51	−4.7

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation. ($100\sigma/M$) divided by 100. It has a different probable error.

In Table VIII is the material on normal women (61 cases) published by Boothby and Sandiford. Here the Bailey prediction is the best, but none of

the differences is by itself significant. The comparison on the relative levels of the standards agrees accurately with that on the previous body of data.

The standards built on the DuBois height-weight surface area formula give the best agreement with the data for women, the Krogh standard coming second, and the Mayo third.

Standards for Children

Standards for children are in an even more confused and divergent condition than standards for adults. In Table IX is listed the comparison of

Table IX

Prediction Value of Various Standards on Data of Benedict and Talbot Common to All (Boys, 27 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Benedict-Talbot interpolated
	Per cent	Per cent	Per cent	
Benedict-Talbot weight (interpolated)	6.01±.551	±4.05	100.7±.78	0.0
Dreyer (observed weight)	6.15±.564	±4.15	113.0±.90	+12.3
Benedict-Talbot weight (as published)	6.19±.568	±4.18	100.3±.81	− 0.4
Power formula for children	6.24±.572	±4.21	97.2±.79	− 3.5
Harris-Benedict	6.50±.596	±4.38	97.7±.82	− 3.0
Mayo	6.75±.619	±4.55	112.6±.99	+11.9
General power formula	6.78±.622	±4.57	99.6±.88	− 1.1
Bailey	7.39±.678	±4.98	118.8±1.14	+18.1
Kestner-Knipping	7.80±.716	±5.26	110.9±1.13	+10.2

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different probable error.

the standards on all boys over 5 years of age and over 25 kilograms weight, in the data of Benedict and Talbot (16). The age limit was required by the Mayo, Bailey and Dreyer standards, and the weight limit by Stoner's table of the Dreyer standard (6), which does not extend below 25 kilograms weight. When the Benedict-Talbot weight standard (16) is interpolated by tenth of kilogram steps, its accuracy is slightly increased. The author's power formula for children ($2311\ \text{Age}^{.304}\ \text{Ht.}^{-.638}\ \text{Wt.}^{.484}$) gives a slightly less accurate prediction than the Benedict-Talbot weight standard.

Table X contains the comparisons on the data of Rosenblüth (17). In all of the tables here published on children, successive determinations on

the same child at intervals of more than a year are treated as separate cases. The standard of Dreyer is here the best, and the Nobel-Rosenblüth (17) is included merely to show how bad it is. The Kestner-Knipping (18)

TABLE X

PREDICTION VALUE OF VARIOUS STANDARDS ON DATA OF ROSENBLÜTH
(Boys, 11 years to 19 years, 84 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Benedict-Talbot
	Per cent	Per cent	Per cent	
Dreyer (observed weight)	6.17±.321	±4.16	89.8±.45	+4.5
Mayo	6.68±.347	±4.50	95.2±.49	+9.9
Bailey	6.87±.358	±4.64	94.5±.51	+9.2
Benedict-Talbot weight	7.34±.381	±4.95	85.3±.54	0.0
Kestner-Knipping	7.54±.393	±5.09	91.4±.56	+7.8
Nobel-Rosenblüth	8.96±.466	±6.04	83.6±.66	−1.7

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different probable error.

TABLE XI

PREDICTION VALUE OF VARIOUS STANDARDS ON DATA OF BENEDICT AND TALBOT COMMON TO ALL EXCEPT DREYER (Girls, 25 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Benedict-Talbot interpolated
	Per cent	Per cent	Per cent	
Benedict-Talbot weight (interpolated)	5.30±.505	±3.57	102.0±.73	0.0
Benedict-Talbot weight (as published)	6.04±.576	±4.07	101.4±.83	− .6
Benedict height	6.06±.578	±4.08	99.9±.82	−1.6
Kestner-Knipping	6.38±.608	±4.30	117.1±1.01	+15.6
Mayo	6.48±.618	±4.37	119.9±1.05	+17.9
Bailey	6.60±.629	±4.45	116.6±1.04	+14.6

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different probable error.

standard shows up poorly on both tables. We must regard the Dreyer as the best standard for boys, with the Mayo standing second, if we accept this evidence.

Tables XI, XII (19) and XIII (20) contain comparisons of the available

data on girls. The Mayo, Bailey and Dreyer standards stand out as the best, in this material. The wide differences of mean level (about 20 per cent on each table) should be noted.

TABLE XII

PREDICTION VALUE OF VARIOUS STANDARDS ON DATA OF BLUNT, TILT, MCLAUGHLIN AND GUNN
(Girls, 7 to 18 years, 54 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Benedict-Talbot interpolated
	Per cent	Per cent	Per cent	
Mayo	7.43±.484	±5.01	104.0±.68	+ 9.6
Bailey	7.45±.485	±5.02	108.3±.69	+13.9
Kestner-Knipping	8.42±.548	±5.68	101.8±.77	+ 7.4
Dreyer (observed weight)	8.60±.580	±5.80	99.9±.82	+ 5.5
Benedict-Talbot weight	9.82±.640	±6.62	94.4±.90	0.0
Benedict height and Benedict age and weight	10.46±.681	±7.01	91.0±.96	−3.4

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different probable error.

TABLE XIII

PREDICTION VALUE OF VARIOUS STANDARDS ON DATA OF MACLEOD
(Girls 10 to 14, 63 cases)

Standard	Standard deviation*	Probable error*	Mean	Mean referred to Benedict-Talbot
	Per cent	Per cent	Per cent	
Mayo	10.92±.657	±7.37	111.3±1.03	+15.7
Dreyer (observed weight)	10.98±.661	±7.41	106.6±1.00	+11.0
Bailey	11.21±.674	±7.56	115.9±1.10	+20.3
Kestner-Knipping	11.49±.691	±7.75	105.3±1.03	+ 9.7
Benedict-Talbot	12.61±.758	±8.50	95.6±1.03	0.0

* Corrected for a mean of 100%. This procedure makes the standard deviation numerically equal to the coefficient of variation ($100\sigma/M$) divided by 100. It has a different porbable error.

THE SELECTION OF NORMAL STANDARDS

There are here presented three power formulae for the prediction of normal basal metabolism, one for use on adult males, one on male children of two years and over, and a third which endeavors to predict the basal metab-

olism of both adult males and male children. Inasmuch as he has been able to demonstrate no definite advantage of these formulae over those in use, the writer regretfully advises no one to adopt these formulae as standards. Since these formulae are but typical of an almost unlimited number of possible other formulae of about the same prediction value he would respectfully suggest to other similarily ambitious individuals that they also restrain themselves until such time as they are able to present statistically significant data to the effect that the new formula marks a real advance in prediction accuracy.

There are several criteria for the selection of a normal standard. The first obviously is prediction accuracy, which will be *relative* prediction accuracy. A second is range of applicability, in that one standard is preferable to several. A third criterion is simplicity of application. Then also, since uniformity is desirable, there is a certain seniority of accepted and established standards to be considered. Still another desirable feature for a basal metabolism standard is approximate agreement in regard to the mean metabolic rate for comparable groups of normal men and women.

In the opinion of the writer, the Dreyer standards based upon observed weight meet the criteria outlined, on the whole, the best. They are the simplest of application in that a single table (5, 6) can be used and the measurement of height is rendered unnecessary. They have a wide range of applicability (from 5 years of age on). They have, on the whole, the best relative prediction accuracy of any standard on the material presented. They have a degree of established usage, particularly in England. The substitution of calculated weight for actual weight in the Dreyer standard is to be condemned. This practice has no observable advantage. According to the available evidence it noticeably diminishes the relative prediction accuracy of the Dreyer standard, as might be expected from the fact that it substitutes inaccurate measurements for accurate ones. It sacrifices the simplicity of the Dreyer standard, and makes it the most complicated of all, and by this token increases the likelihood of errors of calculation.

The Mayo standards are the nearest competitors to the Dreyer standards, but are more complex. They have equal range of applicability, and while not widely used at present, are built upon the DuBois height-weight formula for body surface, which has a wide usage.

The Harris-Benedict standards give excellent agreement results for adults, but necessitate other standards for children, with the confusion this introduces.

In the face of the evidence, the support of accumulated data seems a doubtful argument to commend a continuance in the use of the DuBois

standard. This historic standard seems to have fewer advantages and more disadvantages than those considered above.

None of these standards may be considered a close approach to true accuracy, and the variation between them in individual cases is not infrequently considerable. It would undoubtedly be good policy in all border-

TABLE XIV

CORRECTION FACTORS FOR COMPARISON OF STANDARDS FOR MEN

Rate compared	Rate compared with					
	Krogh	Dreyer (observed weight)	Harris-Benedict	Aub-DuBois	Bailey	Mayo
Krogh	0%	−2%	−2%	−7%	−7%	−9%
Dreyer (obs'd weight)	+2%	0%	0%	−5%	−5%	−7%
Harris-Benedict	+2%	0%	0%	−5%	−5%	−7%
Aub-DuBois	+7%	+5%	+5%	0%	0%	−2%
Bailey	+7%	+5%	+5%	0%	0%	−2%
Mayo	+9%	+7%	+7%	+2%	+2%	0%

TABLE XV

CORRECTION FACTORS FOR COMPARISON OF STANDARDS FOR WOMEN

Rate compared	Rate compared with					
	Krogh	Dreyer (observed weight)	Harris-Benedict	Mayo	Aub-DuBois	Bailey
Krogh	0%	−1%	−2%	−5%	−6%	−6%
Dreyer (obs'd weight)	+1%	0%	−1%	−4%	−5%	−5%
Harris-Benedict	+2%	+1%	0%	−3%	−4%	−4%
Mayo	+5%	+4%	+3%	0%	−1%	−1%
Aub-DuBois	+6%	+5%	−4%	+1%	0%	0%
Bailey	+6%	+5%	+4%	+1%	0%	0%

line or doubtful cases to calculate the basal rate according to at least two of these standards. In these cases correction must be made for the difference in the level of the two standards. This can effectively be done by adding a correction factor obtained from Tables XIV-XVII. Since we must recognize that the standards are imperfect we could in this fashion diminish to a certain degree the likelihood of classifying a high or low normal rate as pathological because of error in the standard. For example, let us consider case 40 of Hobson (male, age 15 years, 11 months, height, 162.7 cm., weight 47.0 kg., caloric consumption at the rate of 1,376 calories for 24 hours).

The basal metabolism by the Harris-Benedict standard is +11 per cent. The rate by the Aub-DuBois standard is −10 per cent, and to make this comparable with the Harris-Benedict we have to add 5 per cent (Table XIV) to correct for the difference of means. The Aub-DuBois comparison rate is −10 per cent +5 per cent = −5 per cent and the Dreyer comparison

TABLE XVI

CORRECTION FACTORS FOR COMPARISON OF STANDARDS FOR BOYS

Rate compared	Rate compared with				
	Benedict and Talbot	Dreyer (observed weight)	Kestner and Knipping	Mayo	Bailey
Benedict and Talbot	0%	−6%	−7%	−11%	−11%
Dreyer (obs'd weight)	+ 6%	0%	−1%	− 4%	− 5%
Kestner and Knipping	+ 7%	+1%	0%	− 4%	− 5%
Mayo	+11%	+4%	+4%	0%	− 1%
Bailey	+11%	+5%	+5%	+ 1%	0%

TABLE XVII

CORRECTION FACTORS FOR COMPARISON OF STANDARDS FOR GIRLS

Rate compared	Rate compared with				
	Benedict and Talbot	Dreyer (observed weight)	Kestner and Knipping	Mayo	Bailey
Benedict and Talbot	0%	−8%	−9%	−12%	−16%
Dreyer (obs'd weight)	+ 8%	0%	0%	− 4%	− 9%
Kestner and Knipping	+ 9%	0%	0%	− 3%	− 9%
Mayo	+12%	+4%	+3%	0%	− 4%
Bailey	+16%	+9%	+9%	+ 4%	0%

rate is −8 per cent +0 per cent = −8 per cent. Here, as a rather extreme example, is a difference of from +11 per cent to −8 per cent depending upon the choice of standard even after correction has been made for the differences of mean level. This subject, who, by the Harris-Benedict standard, had a borderline high rate, probably has a normal metabolism.

CONCLUSIONS

1. The empirical test of a basal metabolism standard is its accuracy in the relative prediction of the basal metabolism of normal human beings.

2. For the clinical application of any basal metabolism standard there should be available the mean basal metabolism of a series of normal individuals of the locality, determined under the conditions used for clinical determinations. Such a series should comprise 25 or more individuals.

3. The Dreyer standards based upon observed weight seem on the whole to have the greatest advantages and the least disadvantages. These standards are available in very convenient form in the tables of Stoner (5, 6).

4. In all borderline or doubtful cases the metabolic rate should be calculated by more than one standard, making allowance for the difference in the mean level of the standards. In this procedure one standard based upon the DuBois body surface formula cannot be regarded as a check upon another of the same type.

5. In reporting basal metabolism in the medical literature, the standard used should always be specified.

6. Until such time as there is adequate study of normal human beings to provide definite and statistically significant evidence of the superiority of new standards, this field of investigation is better off without them.

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EFFECT OF VITAMINS A AND D ON RESISTANCE TO INFECTION

By

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THAT laboratory animals in advanced stages of vitamin A deficiency suffer from spontaneous infections is an established fact. Green and Mellanby (1928) found evidence of infection in all but two white rats dying from deficiency of vitamin A. Abscesses at the base of the tongue were the most commonly observed lesion, being present in 72 per cent of such animals examined. Infections of the urinary tract, particularly of the bladder, were very common in Green and Mellanby's series. Mastoid and nasal sinusitis, purulent otitis media, ocular, respiratory and alimentary tract infections occur frequently in vitamin A-starved laboratory animals, apparently because of a lowered resistance to organisms to which the animals were normally exposed. Bacterial and pathological studies of several of these conditions have been made. (Daniels, *et al*, 1923; Werkman, 1923; Goldblatt and Benischek, 1927; Shurly and Turner, 1930; Verder, 1928.)

In contrast to the almost invariable occurrence of infection in animals suffering from deprivation of vitamin A, it is not unusual to encounter the most florid type of rickets, both in the infant and in the experimental animal, unassociated with any apparent bacterial lesion. There is, however, some experimental evidence suggesting lowered resistance to infection in animals deprived of vitamin D.

By decreasing calcium and the antirachitic vitamin in the diet of white rats, Grant (1927) demonstrated a marked increase in their susceptibility to tuberculosis. Eichholz and Kreitman (1928) reported that a much larger proportion of rachitic rats than of normal animals died as the result of a spontaneous paratyphoid epidemic. They also demonstrated greater resistance to injections of pneumococci in white mice fed a rachitic diet supplemented by irradiated ergosterol, in comparison with those receiving the unsupplemented ration.

Robertson (1927) injected rats on a rachitic diet with Gram-negative bacilli isolated from the nose of a normal rat. A smaller percentage of the

animals kept out of doors succumbed to the infection than of those remaining inside. Some increased resistance was also obtained by supplying irradiated ergosterol as the antirachitic agent. For some reason, however, cod liver oil apparently failed to have a similar effect. Hill and Clark (1927) on the other hand, report negative results in an attempt to increase the resistance of rats on an adequate diet, to the inoculation of Type I pneumococci by ultra violet radiation. Ackert and Spindler (1929) were unable to obtain clear-cut evidence that chickens deprived of vitamin D were less resistant to infection by *Ascaris lineata* than were those fed on adequate diets.

We proposed to study further the relation of both vitamin A and vitamin D to infection, endeavoring to obtain evidence of impaired resistance throughout the course of the development of the deficiency. A more accurate demonstration of the influence of these vitamins should be expected before the effects of the depletion became pronounced. It is quite obvious in an experiment of this kind that if animals are infected in the terminal stages of almost any food deficiency, they are apt to succumb readily to the infection.

Experimental

The animals used were four weeks-old white rats reared from mothers on the same adequate stock diet. The young rats of each litter were so distributed that approximately an equal number of litter mates of about the same average weight comprised the test and control groups. They were kept in sterilized wire cages with raised bottoms.

Diets. The vitamin A-free diet (extracted casein 18 per cent, starch 76 per cent, agar 2 per cent and Osborne and Mendel salt mixture 4 per cent) was supplemented each day by 0.5 gram of dry powdered yeast and 0.0015 mg. of irradiated ergosterol in olive oil. In addition to the above diet fed *ad libitum*, the control rats each received 6 drops per diem of a commercial, tested brand of cod liver oil.

The rachitic diet (Steenbock) was made up of ground yellow corn 74 per cent, wheat gluten 20 per cent, calcium carbonate 3 per cent and sodium chloride 1 per cent. The control animals received in addition daily doses of 0.0015 mg. of irradiated ergosterol in olive oil.

The organism. Various pathogenic organisms have been used in similar animal-infection experiments, such as the paratyphoid group, pneumococcus, B. anthracis, B. tuberculosis, B. coli, etc. Since our first attempts were in the direction of producing upper respiratory tract infections, it was thought advisable to obtain an organism naturally found in this location

in the rat. In a previous study, a certain bacillus of the *mucosus capsulatus* group was found to be a common inhabitant in the suppurative lesions of the respiratory tract in vitamin A-deficient rats (Bradford, 1928).

The organism was pathogenic for rats and for other laboratory animals and proved satisfactory for intraperitoneal inoculations in these experiments. Before inoculating each group of animals, the dose was determined by intraperitoneal injections in a series of stock rats of approximately the same age and weight as those to be infected. The infecting dose was taken as that amount of a 1 to 10 dilution of a 24-hour broth culture which just permitted the normal rat to live. It usually ranged between 0.25 cc and 0.4 cc.

Ninety-one rats were used in the experimental series, 46 being put on the vitamin A-free diet, and 45 fed the rachitic ration. Twenty-three rats of the vitamin A group were used as controls and given cod liver oil; likewise 22 of the vitamin D group were given irradiated ergosterol as controls. A group of vitamin A-deficient animals was inoculated along with an equal number of controls respectively, at the end of 4, 6, 8 and 10 weeks upon the experimental diet. The rachitic animals with their controls were divided into three groups which were infected at the end of 4, 6, and 8 weeks respectively.

Post mortem studies. Careful observations of each animal's reaction to the infection and of post mortem findings were made. Those that survived the infecting dose were examined anatomically ten days later. In each animal dying from the infection, acute purulent peritonitis was present. In addition, the lungs, kidneys, spleen and liver showed evidence of acute infection. From the heart's blood and spleen in ten animals cultured, the infecting organism was recovered in pure culture in each case.

In the vitamin A-deficient group, the majority of the test animals infected after 8 to 10 weeks on the diet, showed clinical and anatomical evidences of vitamin A deficiency, such as dry, rough coats, atrophic testes, protruding dried penis and spontaneous infections such as tongue and lung abscesses and purulent sinusitis and otitis. None showed evidence of rickets. In no instance did a control animal show evidence of either vitamin A or D deficiency.

In the rachitic group there was evidence of active rickets in the majority of the animals. In those examined at the end of 6 and 8 weeks, it is interesting to note that spontaneous infections such as characterized the terminal stage of vitamin A-deficient animals were not present. The acute lesions produced by the injected organisms were quite similar. Upon the basis of gross anatomical study, the degree of rickets in the series of sub

groups may be classified as follows: 4 weeks, mild; 6 weeks, moderate; and 8 weeks, severe. The control animals revealed no gross evidence of rickets at autopsy.

Discussion of Results

In Chart 1 graphic representation of the results in the vitamin A-deficient group is given, according to the sub groups infected. In the vitamin

CHART 1.

Vitamin A Deficient Animals.

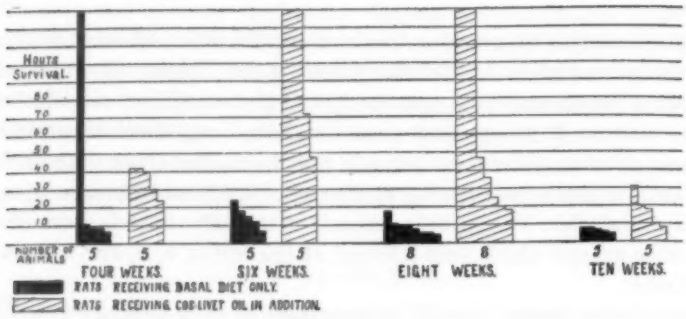
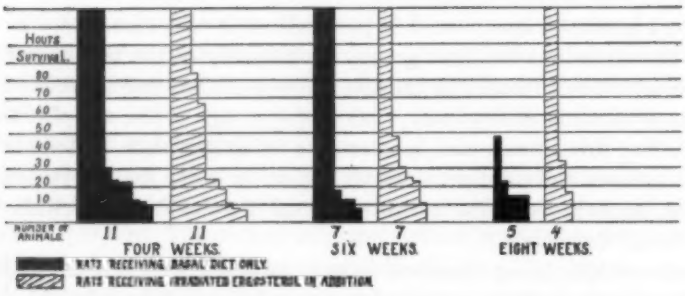


CHART 2.

Vitamin D Deficient Animals.



A-deficient group it is noted that one survived the infection entirely and 22 died. The only survival was in the first sub group infected, which was after four weeks on the experimental diet. The average duration of life after in-

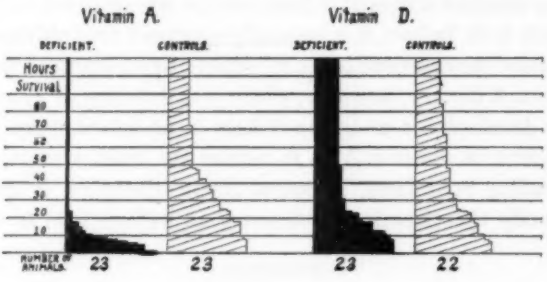
oculation was 10 hours. Of the 23 animals that received the same diet plus cod liver oil (controls) 6 lived and 17 died. The latter survived for an average time of 31 hours.

Chart 2 shows the results obtained among the group of vitamin D-deficient rats. Seven lived and 16 died. Of the controls, 7 lived and 15 died. The rachitic animals survived the inoculation for an average period of 24 hours, while the controls averaged 34 hours.

Chart 3 is a composite graphic comparison of the resistance to infection of the entire groups of deficient animals along with their respective controls.

CHART 3.

Comparative Resistance of Vitamin-deficient Rats to Infection.



As was to be expected from the repeated observations of many infections in animals dying from the lack of vitamin A, the data here presented show a definitely increased susceptibility to infection in the animals fed the vitamin A-free diet, as compared with their controls which received cod liver oil in addition, and also with the vitamin D-deficient group.

Of particular importance, however, is the fact that whereas six to eight weeks on the vitamin A-free diet were required to so deplete the body stores of vitamin A as to result in cessation of growth, yet marked susceptibility to infection was evident as early as the fourth week on the deficient diet. Increased susceptibility to infection is apparently an early manifestation of a dietary low in vitamin A, having marked influence before the physical signs of deficiency are present. The animals in the group inoculated at four weeks were seemingly healthy, active specimens, maintaining a rate of growth not greatly below the controls receiving cod liver oil. This point is of especial clinical significance since classical signs of vitamin A deficiency, such as xerophthalmia, are rarely seen in this country; yet it is

quite probable that relative degrees of vitamin A deficiency exist, predisposing the individual to various types of infection.

The work of Sherman and Burtis (1928) suggests such susceptibility as an early sign of vitamin A depletion. They find further, "that the influence of the level of intake upon the incidence of infection may be pronounced and long continued even when the differences of diet are no greater than may readily occur within the range of ordinary normal or adequate nutrition."

In regard to the vitamin D-deficient animals, the results obtained at the end of 4 and 6 weeks do not show definite evidences of an anti-infective influence of vitamin D. Fewer rachitic animals than controls survived in the group inoculated at the end of eight weeks on the experimental diet, by which time the rickets was well advanced. Undoubtedly an animal or an infant in the terminal stages of rickets may be less resistant to infection or even succumb to it. In fact, it is generally supposed that children suffering from rickets are very susceptible to infections, particularly those of the respiratory tract. A dietary, however, which is so deficient in vitamin D as to lead to the development of active rickets in an infant, very probably is also so deficient in vitamin A as to render the child a more susceptible host to invading organisms. Therapeutically, therefore, it seems important to remedy the vitamin A-deficiency as well as that of vitamin D.

Summary

1. Young white rats were inoculated by intraperitoneal injection of a standard suspension of organisms after 4, 6, 8 and 10 weeks on a vitamin A-free diet. Markedly decreased resistance to such infection, compared with controls receiving cod liver oil, was demonstrated before other signs of vitamin A deficiency appeared.

2. No such susceptibility to similar inoculations was found in young rats on a diet deficient in vitamin D, compared to controls protected by irradiated ergosterol.

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STUDIES IN PROTEIN NUTRITION OF THE CHICK

I. THE INFLUENCE OF DIFFERENT PROTEIN CONCENTRATES ON THE GROWTH OF BABY CHICKS, WHEN FED AS THE SOURCE OF PROTEIN IN VARIOUS SIMPLIFIED DIETS

By

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FOR the past three years we have been engaged in investigations concerning the comparative value of various commercial protein concentrates as nitrogenous feeds in the poultry ration, particularly in regard to their influence on reproduction. In the course of this work it became evident that to further our knowledge of the dietary requirements for reproduction in the chick, we had to conduct our feeding trials under more carefully controlled conditions. At this time several workers had reported the successful growing of chicks under laboratory conditions, the food requirements being covered by synthetic diets. We decided to avail ourselves of this information and to determine first the comparative growth value of these protein supplements when fed as the sole source of protein in an otherwise synthetic diet. The present paper presents some of the difficulties which we have encountered, showing that our present concept of synthetic diets for chicks is quite inadequate. The protein feeding stuffs studied were buttermilk powder, fish meal, cod-liver meal and two abattoir by-products, meat meal and tankage.

Experimental

Our objective was to procure a synthetic basal diet, which, while satisfying the vitamin requirements, etc. of the chick, contained the minimum amount of protein and to supplement this diet with equal amounts of crude protein from the different protein supplements. A review of the literature revealed that our greatest difficulty in using a synthetic basal diet was to prevent the incidence of leg-weakness. Hart, Halpin and Steenbock (1920) found that lack of a suitable roughage in the diet was the major cause of leg-weakness and that normal chicks could be grown on a

synthetic diet composed of butter fat 15; dried yeast 15; salts 5; casein 18; dextrin 37 and paper 10; the latter being ground into the diet. Later, Hart, Steenbock and Lepkovsky (1924) reported that more uniform growth could be obtained by replacing the butter fat with 5 per cent of an ether extract of saponified cod-liver oil. In our first series of experiments, a modification of this synthetic diet was used as our basal diet.

First Series

The total fat content of these protein supplements is not only extremely variable (see Table I) but the vitamin A and vitamin D contents are

Table I

Percentage Composition of Protein Supplements

	Meat Meal	Tankage	Fish Meal	Cod-Liver Meal	Buttermilk Powder
Moisture	3.84	5.89	5.90	3.4	7.62
Crude protein (N×6.25)	55.90	56.70	75.77	41.9	34.80
Ether extract	14.58	10.91	3.86	44.1	5.31
Crude fibre	1.22	1.79	0.23	0.3	—
Lactose	—	—	—	—	37.91
Lactic acid	—	—	—	—	5.82
Total ash	19.51	20.28	17.63	2.83	8.47
Silica	0.44	0.68	1.90	0.05	.20
P_2O_5	6.85	6.72	5.55	0.64	1.48
CaO	8.55	8.74	5.97	0.15	1.23
MgO	0.59	0.61	0.88	0.09	.22
Na_2O	1.02	1.55	1.81	0.67	2.25
K_2O	0.44	0.88	0.48	0.25	.41
Fe_2O_3	0.04	0.15	0.016	0.009	0.007
Cu(Mg/Kg)	5.4	7.2	4.2	13.35	3.8

also quite variable in the different materials. As we were particularly concerned with a comparison of the protein of these materials, we have freed them from the variable amount of fat which they contain by extracting with ether in a large copper Soxhlet extractor (Bryant, 1929). This procedure was adopted in all the experiments described. At the time these experiments were begun the inorganic analysis (Table I) had not been completed. The amount of ether extracted meal, required to give 18 per cent crude protein, added to the diet the largest amount of ash, 8.9, per cent so that it was decided to bring the ash content of all the diets to this level using the ash of fish meal. We felt, in view of the high value which Orr (1925) has placed on the inorganic constituents of fish meal, that

any difference in the composition of the ash of the different protein supplements which might be a limiting factor in growth would be overcome by this procedure. The results of the ash analysis when completed showed that this assumption was justified.

The following "basal" synthetic diet was supplemented by 18 per cent crude protein from each of the protein concentrates.

	Per cent
Yeast extract	10
Butter oil	10
Cod liver oil (saponified)	2
Paper pulp	10
Fish meal ash	8.9 total ash
Dextrin	100

Ultra-violet light—10 min. twice weekly.

Our endeavour was to obtain a source of vitamin B which would not necessitate the addition to the diets of large amounts of extraneous nitrogenous compounds. An alcoholic extract of dried brewer's yeast, prepared according to Hartwell (1922), was used. The saponification of the cod-liver oil and the ether extraction of the unsaponifiable residue was carried out according to Steenbock, Jones and Hart (1923). A high grade medicinal cod-liver oil was used and an amount of the unsaponifiable residue in ether equivalent to 2 per cent of the original oil was added to the diets and the ether evaporated off. A high grade paper pulp was dried and ground in a Wiley mill and then 10 per cent of the ground paper was reground into the diets.

The experiment was commenced with day-old barred rock chicks, thirty in each group, housed in Carrick Brooders (1925) in the laboratory. At the end of ten days the number in each group was reduced to twenty by weeding out those chicks showing evidence of constitutional weakness. Distilled water was before the birds at all times and the diets were prepared in amounts to last approximately one week. Post mortem examination was made on all birds that died and hemoglobin determinations (Newcomer) were made on all the birds which survived the six weeks duration of the experiment.

NOTES

GROUP I. *Buttermilk Powder:* Chicks hatched May 21; feeding commenced May 24. Throughout the experiment these were strong healthy chicks. Their feces were extremely watery and by June 1 they were extremely dirty. On June 2, several of the heavier chicks showed deformity of the toes, the digits of the foot becoming crooked. On June 5, the ultra violet irradiation was increased to 20 min. without effect on the toe deformity. On June 9, the biggest chicks developed leg-weakness, walking with a stilted gait, their legs bending at the hock joint. The joints of the metatarsus and

the digits became puffy. By June 11, three birds were paralysed in the legs and wings—the joints being badly swollen and having a bluish color. By June 18 the yeast extract was increased to 15 per cent and four birds were receiving 40 mg. yeast vitamin B concentrate (Harris) per day, in a gelatin capsule. The birds were only prevented from eating heartily by complete paralysis. On July 2 the experiment was concluded with 14 birds surviving, all more or less suffering from leg-weakness and appearing to be anemic. The average hemoglobin content of the blood was 8.5 gms. per cent with individual variations from 7.6 to 9.4 gms. per cent. The blood volume was low.

GROUP II. *Meat Meal:* Chicks hatched May 22; feeding commenced May 26. Little or no growth was made by this group. By May 31 the chicks showed an apparent loss of vigor, preferring to remain under the hover. On June 13 there were only nine birds left and these had made little or no growth. On June 29 the experiment was discontinued with one bird surviving. Certain birds in this group showing signs of failing were also given 40 mg. per day yeast vitamin B concentrate without effect. In general, the post mortems showed the liver to be badly congested, the kidneys hypertrophied with many small hemorrhages and the gall bladder small, transparent and gelatinous. The ureters and cloaca were almost plugged with urates. One outstanding pathological condition noted in many cases was that the lining membrane of the gizzard was eroded and exfoliated medially.

GROUP III. *Tankage:* Chicks hatched May 22; feeding commenced May 26. The results with this group were very much the same as with Group II. The same dull, unthrifty appearance with little or no growth. Cannibalism, toe and feather picking, was practiced to a large degree. The last chick died on June 27. In general the post mortem conditions were as in Group II, but the eroded and exfoliated condition of the lining membrane of the gizzard was more severe and more prevalent. In some cases the lining membrane of the gizzard was practically destroyed; in others it was darkened, shrunken and appeared as if slightly burned, suggesting that something toxic was present in meat meal and particularly tankage.

GROUP IV. *Fish Meal:* Chicks hatched May 23; feeding commenced May 26. During the first two weeks these chicks were the best of the entire experiment. Around June 20 a general dullness manifested itself and the mortality was great, all the chicks being very anemic. What appeared to be big healthy chicks died and were found to be extremely anemic, the blood volume being also extremely low. The experiment was concluded on June 30, chloroforming the last remaining bird. From June 13, on first signs of failing, several chicks received 40 mg. yeast vitamin B concentrate per day with no apparent effect. The outstanding observation in the post mortems was the extreme general anemia. Hemoglobin determinations on a number of birds were made, the highest being 7.7 gms. per cent and the average 6.3 gms. per cent. A 1 per cent $CuSO_4$ solution was administered to a number of birds by pipette, without any beneficial result.

GROUP V. *Cod Liver Meal:* Chicks hatched May 23; feeding commenced May 26. Up to June 13 this group did very well but from this date the birds developed chronic diarrhoea. On June 23, 5 per cent charcoal was added to the diet and as a result the feces were greatly improved. There were fewer losses in this group than in any other, excepting Group I. On July 3 this experiment was concluded with 10 chicks surviving. At this time, while they were growing fairly well, they did not seem to be feathering properly and had rather a dull grey color. Hemoglobin determinations were made on the surviving birds, the average result being 10.71 gms. per cent which was higher than in any other group. The chicks seemed to have a remarkably great blood volume compared to the other groups. The mortality was confined to chicks showing extreme ante-mortem diarrhoea.

Chart 1 shows the average growth in grams obtained with each group. The weekly mortality is also given. It will be seen that the growth on none of these diets was satisfactory. While fair growth was made by group I, the incidence of extreme leg-weakness showed this diet to be far from satisfactory. The complete failure of the meat meal and tankage groups was very surprising.

Palmer and Kennedy (1928) have reported that vitamin G (B_2) is only very slightly soluble in water or 90 per cent ethyl alcohol. It seemed then not improbable that the yeast extract which we used may have contained very little of the growth factor present in the yeast and this might account for the unsatisfactory growth in this experiment. We then repeated group I replacing the 10 per cent yeast extract by an equivalent amount of the untreated brewer's yeast, *i.e.*, Group VI. To determine to what extent the yeast protein supplemented the protein of this diet we also put one group on the same diet as Group VI except that the buttermilk powder was replaced by an equivalent amount of crude protein from pure edible

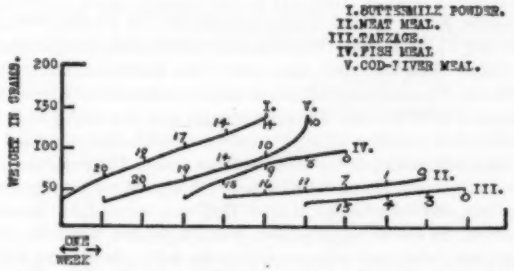

Chart 1.—Showing the growth with the different proteins supplementing a basal diet composed of yeast extract 10%; butter oil 10%; cod-liver oil (sap.) 2%; paper pulp 10%; fish meal ash to 8.9%; total ash, and dextrin to 100.

Note. In these, and in all other growth curves recorded in this paper, the arabic figures on each curve give the number of chicks living at each particular time.

gelatin, *i.e.*, Group VII. A third group was fed the same diet as Group VI except that in place of brewer's yeast, filtered tomato juice, concentrated in vacuo at 140°F. was used as the source of vitamin B an arbitrary amount (the equivalent of 25 cc. of the original unconcentrated juice per bird per day), being given, *i.e.*, Group VIII. The remainder of the diet and the conditions under which this experiment were conducted, as regards the number of chicks, etc., were the same as has been described.

NOTES

GROUP VI. *Buttermilk Powder, Brewer's Yeast:* This group made better growth but developed leg-weakness to a greater extent and in even greater severity than did Group I. On the first signs of approaching leg-weakness, as evidenced by the crooked toes, the amount of yeast was increased to 15 per cent and a few days later to 20 per cent, replacing an equivalent amount of dextrin, without delaying the onset of the leg paralysis. The three mortalities were chicks which were completely paralysed, death being due to the inability of the birds to move about and feed. The average hemoglobin content of the blood of the surviving birds was 9.23 gms. per cent with variations from 8.5 to 13.6 gms. per cent. It was difficult to obtain blood from the brachial artery as the blood volume was extremely low. Ash determinations on the dry, fat-free, femurs and tibias and blood

serum calcium and phosphorus determinations were made on six chicks from this group which were suffering from extreme leg-weakness with the following results:

Sample No.	Percentage Ash		Calcium* (Mg. per 100 cc.)	Phosphorus** (Mg. per 100 cc.)
	Femur	Tibia		
1.	52.84	52.99	14.9	6.10
2.	55.98	53.58	14.4	6.37
3.	62.56	58.24	13.8	6.62
4.	56.23	53.53	14.4	5.83
5.	62.25	51.05	13.3	5.88
6.	58.68	—	14.9	6.50

* Determined by the Clark-Collip modification of the Kramer-Tisdall method (1925).
** Determined by the method of Fiske and Subbarow (1925).

GROUP VII. *Gelatin, Brewer's Yeast:* Chicks hatched May 24. Feeding commenced May 26. By June 8 there were only 11 chicks left in the group and these had not, in some cases, even maintained their original weights. Those that died, apparently from starvation, had practically completely absorbed the yolk sac. The mortality and loss in weight continued until June 13 when the amount of yeast was increased to 15 per cent. The improvement was very slight; while some made a little gain in weight, others lost weight and died. The extreme cannibalism, toe, head and plumage picking, which had been outstanding in the group still continued. The experiment was concluded on July 3, with two chicks living. Hartwell (1926) found that yeast extract supplemented gelatin in a synthetic diet for rats, and Osborne and Mendel (1919) were successful in growing rats on a diet in which yeast furnished the sole source of protein. Funk, Lyle, and McCaskey (1916), however, assert that "a large part of the yeast nitrogen apparently has no food value, it being badly assimilated by rats."

GROUP VIII. *Buttermilk, Powder—Tomato Juice:* Chicks hatched May 24. Feeding commenced May 26. At the outset it was found impossible to feed the tomato juice by placing it before the chicks as they would not consume very much and when allowed access to water they drank as if they had been thirsty for some time. The concentrated juice was then mixed into the diet. On May 29, 5 chicks died, all having greatly enlarged abdomens and showing a post-mortem condition of general oedema. A change was made, incorporating the original unconcentrated tomato juice into the diet in an amount to give about 20 cc. per bird per day. From this time the birds made fairly good gains in weight, but by June 27 developed leg-weakness just as did Groups I and VI. On July 3 the experiment was discontinued with one bird living. The mortality was either the result of general oedema or extreme leg-weakness.

In Chart 2 is shown the average growth in grams and the weekly mortality of these three groups compared with Group I. The growth in Group I with yeast extract was not as good as the growth with Group VI with untreated medicinal brewer's yeast. It seemed probable that the results obtained with fish meal, meat meal, tankage and cod-liver meal may have been similarly affected by a lack of vitamin G (B_2) in the yeast extract used. It was, therefore, decided to repeat the experiments with these four protein supplements using the unextracted dried brewer's yeast.

SECOND SERIES

In repeating the experiments with Groups II to V inclusive, the following changes were made in the diets. Each group received 15 per cent of

dried brewer's yeast. The ether extract of the unsaponifiable residue of cod-liver oil was dispensed with and all groups received 5 per cent medicinal cod-liver oil. The experiment commenced on July 31 with selected, day-old Barred Rock chicks, thirty in each group. Each group was further divided at feeding time into three lots of ten chicks; an arbitrary change was made in the amount of crude protein from the protein concentrate and in the fibre content of the diets in each lot, as follows:

Lot I	Lot II	Lot III
18% Crude protein	12% Crude protein	18% Crude protein
10% Paper pulp	10% Paper pulp	5% Paper pulp

The composition of the remainder of the diet, except for the change in the yeast and cod-liver oil, was as in the first series of experiments.

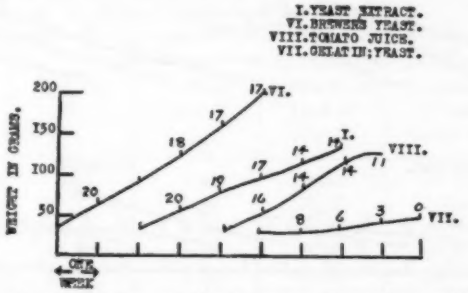

Chart 2.—Showing the comparative growth with buttermilk powder and different sources of vitamin B, and the growth with gelatin and brewer's yeast.

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GROUP IX. *Tankage:* The growth of the chicks in this group showed very little improvement from that obtained with Group III, particularly in the case of lots I and II. There was a slight improvement in lot III where the fibre content of the diet was halved. Cannibalism was just as bad as in Group II and the post mortem conditions were again characteristic of starvation and general emaciation.

GROUP X. *Meat Meal:* Extremely poor results were obtained with this group, there being little or no growth with all three lots and the mortality was just as bad as in Group II. The post mortem conditions were, as before, characteristic of complete starvation. The change in the amount of meat meal and fibre in the diet had little or no effect. It was characteristic of this group that they weakened very quickly, showed little or no interest in their food and then died.

GROUP XI. *Fish Meal:* The results obtained with this group were much the same as with Group IV. For a time the chicks did extremely well, but were always pale and anemic looking. From the fourth week we continued to lose several chicks each day. A big, healthy chick would appear quite normal and then, within a few hours, become suddenly weak and unsteady on its legs and in a short time fall over, stiffen out and die. A typical post mortem examination of such a mortality revealed the viscera to be colorless, the kidneys, lungs, and liver being very pale and the heart seemingly bloodless. Numerous pin-point hemorrhages in the kidneys and an excess of water in the ureters and cloaca were noticed. In a few cases there were great blotches of hemorrhages all along

the left side and left leg, particularly about the femur. This group received $CuSO_4$ daily in their drinking water and in many cases daily capsules containing 40 mg. yeast vitamin B concentrate (Harris) were given. In general the chicks seemed to make excellent growth to a certain point and then die in a very anemic condition. There was little or no difference in the different lots in this group.

GROUP XII. *Cod Liver Meal:* The results obtained with this group were disappointing and in direct variance with those obtained with Group V. The growth in all lots was poor and the mortality particularly heavy. There was little or no difference in the three lots.

In Chart 3 is shown the average growth in grams and the weekly mortality of these three groups. The failure of these protein supplements in this type of nutritional experiment is hard to explain. Since fair growth was obtained with buttermilk powder, it may be that the other protein

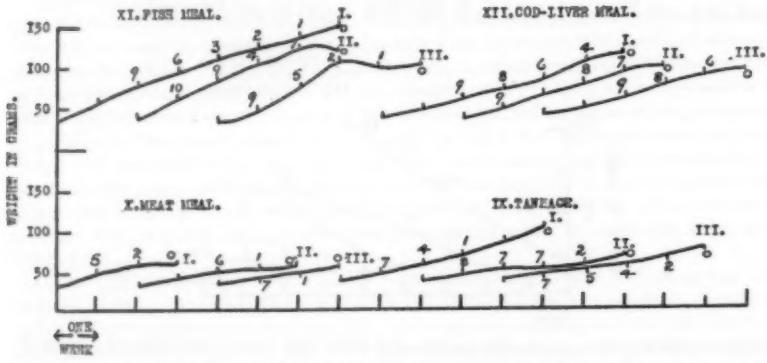

CHART 3.—Showing the effect of varying the amount of protein and fibre in the different diets. Each group was divided into three lots, the same basal diet being supplemented with; I. 18% protein and 10% paper; II. 12% protein and 10% paper; 18% protein and 5% paper.

concentrates, particularly meat meal and tankage, are deficient in one or more essential amino acids. There was evidence, however, that in the case of tankage a change in the physical condition of the diet by reducing the amount of fibre was slightly beneficial. Palatability and digestibility may have been to some extent a limiting factor. Waterman and Jones (1921–1922) have described a method for determining the digestibility of proteins by pepsin and trypsin *in vitro*, the results of which correlate with the results of growth experiments with rats. We have applied this method to these protein concentrates; the results, however, (Table II) did not parallel the results of the growth experiments with chicks and are of little value in explaining the nutritional behaviour of these proteins.

We consistently observed that the chicks in the tankage and meat meal groups seemed very constipated, while the chicks receiving buttermilk

powder seemed extremely laxative. It was thought that if the tankage and meat meal diets could be made more laxative better growth might be obtained. Replacing the dextrin of the meat meal and tankage diets by an amount of lactose and lactic acid, equivalent to that in the buttermilk powder diet, while having a pronounced laxative effect on the chicks, gave no better growth and the mortality was just as great as ever.

THIRD SERIES

It would seem that since leg-weakness developed in the buttermilk powder group, where otherwise growth was fairly satisfactory, the basal

TABLE II

COMPARATIVE DIGESTIBILITY OF PROTEIN CONCENTRATES AS DETERMINED IN VITRO BY THE METHOD OF WATERMAN AND JONES

	% Total N.	% Total amino N.	% Digested amino N. on basis of Total N.	% Digested N. on basis of Total amino N.	% of Total N. as amino N.
Buttermilk powder.	4.53	3.46	31.61	41.39	76.36
Tankage.....	11.91	7.68	14.82	22.98	64.49
Meat meal.....	10.32	6.56	26.31	41.37	63.61
Fish meal.....	12.89	7.97	38.40	52.09	61.83
Cod-liver meal....	10.79	6.81	12.69	20.09	63.03

diet which we have been using was not adequate. In view of this fact we hesitated to draw any conclusions from the results with the other protein supplements. That there is a definite relationship between the protein and vitamin B of the diet has been suggested by the experiments of Plimmer *et. al.* (1927) with chicks, and by those of Hartwell (1928) with rats. By varying the ratio of buttermilk powder to brewer's yeast in the diet used with Group VI, an attempt was made to prevent the occurrence of leg-weakness. As only a few pens were available at the time, only a limited number of arbitrary levels of protein to yeast were tried, as follows:

GROUP XIII.	Ratio Protein : Yeast
Lot I, 15% protein from buttermilk powder: 15% yeast	1 : 1
Lot II, 12.5% protein from buttermilk powder: 15% yeast	1 : 1.3
Lot III, 10.0% protein from buttermilk powder: 15% yeast	1 : 1.5
Lot IV, 10.0% protein from buttermilk powder: 10% yeast	1 : 1
Lot V, 7.5% protein from buttermilk powder: 15% yeast	1 : 2
Lot VI, 7.5% protein from buttermilk powder: 10% yeast	1 : 1.3

The basal synthetic diet was the same as previously described with the buttermilk powder Groups I and VI, except that the amount of medicinal cod-liver oil was increased to 5 per cent. The lactose and lactic acid content was kept the same in all the diets by adding varying amounts to the basal diet depending upon the amount of buttermilk powder. The experiment was commenced with fifteen one-day old barred rock chicks in each lot and was conducted under the conditions already described.

Chart 4 shows the average growth in grams of each of the lots for the six weeks duration of the experiment. It was found that 15 per cent protein from buttermilk powder was the minimum from this particular source which would give reasonable growth comparable with Group VI receiving 18 per cent crude protein from buttermilk powder. Less than 15 per cent protein from buttermilk powder gave distinctly subnormal growth. With

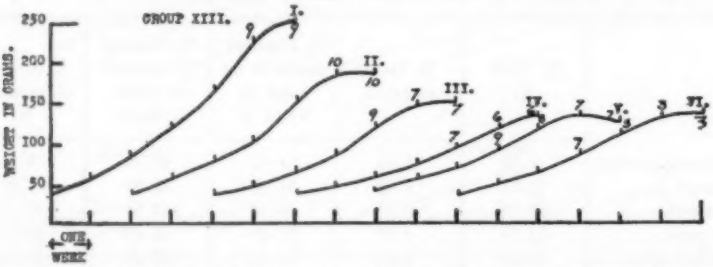

CHART 4.—Showing the effect of the rate of growth of varying the amount of protein (buttermilk powder) and yeast in the diet. I. 15% protein; 15% yeast. II. 12.5% protein; 15% yeast. III. 10% protein; 15% yeast. IV. 10% protein; 10% yeast. V. 7.5% protein; 15% yeast. VI. 7.5% protein; 10% yeast.

lots 1 and 2 where the ratio of protein to yeast was 1:1 and 1:1.3 respectively leg-weakness was just as prevalent as was our previous experience with Groups I and VI. With the lower protein lots even where the protein:yeast ratio was 1:2 (lot 5) leg-weakness still developed although much less severely than in the higher protein groups, lot 1 and 2. This was consistent with our previous finding in Groups I and VI that individual birds making the greatest gain in weight developed severe leg-weakness before the slower growing individuals.

Hogan and Shrewsbury (1930) have reported that good growth and freedom from leg-weakness was procured only when their simplified casein diet was fortified by 40 per cent of yeast. Jack (1926) likewise found that in using synthetic diets with chicks, leg-weakness could be prevented only by increasing the amount of brewer's yeast away beyond what might reasonably be considered the optimum vitamin B requirements of the chicks.

Fourth Series

So far, it had not been found possible to grow normal chicks with a diet in which, excepting for the brewer's yeast, the protein was entirely of animal origin. Plimmer, *et. al.* (1927) have reared chicks successfully on a diet composed of fish meal 15; dried yeast, 20; cod-liver oil, 1; and white rice, 90 parts. This diet is not essentially different from those used by us, *e.g.*, Group XI, except that instead of white rice we have used dextrin, as a source of carbohydrate and ground paper pulp as roughage. When the fish meal was increased to 20 parts, the chicks developed leg-weakness which the authors suggested was due to the kind of protein, as previously they had reared first-class birds using as high a proportion of milk proteins. (See Plimmer and Rosedale (1922.)) In these experiments using caseinogen and dried whey, however, the remainder of the diet was not the same as used in 1927, *e.g.*, oatmeal was used instead of white rice. Believing that our failure to grow normal chicks was associated with the composition of the basal synthetic diet, either in the use of dried yeast, paper or dextrin, we next repeated these feeding trials with the different fat-free protein concentrates, supplementing the following basal diet:

Marmite	15%
Cod-liver oil	3%
Iodized salt*	1%
Bone meal** to	4.36% total ash
White rice to	100.

* Early in the experiment the use of the iodized salt was discontinued. We were not aware that the NaCl content of the marmite was already so great. A number of the baby chicks developed oedema which, however, ceased as soon as the extra salt was removed from the diet.

** Each of the protein supplements was added to the different diets in an amount to supply the equivalent of 10 per cent crude protein (N×6.25). The ash content of the diets was then found to vary within narrow limits. The total ash content of all the diets was brought to the same level 4.36 per cent by adding bone ash. The largest amount added was in the buttermilk powder diet and this was only 0.33 per cent.

It was only necessary to vary the amount of white rice in the different diets within narrow limits, *i.e.*, 65.8 to 67.3 per cent except in the buttermilk powder diet Group XV. The protein content of the buttermilk powder was much less than that of the other protein supplements, so a larger amount of buttermilk powder was required to add 10 per cent protein to the diet and therefore, the amount of white rice was proportionately reduced to 49.3 per cent. The use of tankage as a protein supplement was discontinued. To obtain some idea as to whether the protein of the marmite and white rice was of good quality, Group XVIII was fed

the basal diet with edible gelatin as the source of protein. Again as a check on the marmite used, two groups were given 10 per cent of protein from casein with the same basal diet as before, except that group XIX received 15 per cent marmite while group XX received 15 per cent dried brewer's yeast. With the gelatin and the two casein diets 4 per cent mineral mixture (See Hart *et. al.* (1920)) was added.

The experiment was commenced with over thirty barred rock chicks, one day old, in each group, housed under the conditions already described. At the end of the first week the numbers were reduced to 25 chicks in each group, discarding those showing any evidence of constitutional weakness. Each group received daily ultra-violet irradiation at a height of 24 inches for 10 minutes.

NOTES

GROUP XIV. *Buttermilk Powder:* The growth of these chicks was excellent but throughout the experiment they always had a peculiar appearance. The feathers were moist and ruffled, giving the chicks a wet appearance in the region of the breast and head. The six chicks which died during the first three weeks were all suffering from oedema, the pericardium being filled with a straw-yellow, viscous fluid.

GROUP XV. *Meat Meal:* While the growth of these chicks throughout the experiment was good, the mortality was heavy. Four chicks were lost as the result of hemorrhage on inserting the identification wing bands in the web of the wing; the chicks bleeding slowly for from 12 to 48 hours before death.

GROUP XVI. *Fish Meal:* Twenty chicks were lost in this group during the first four weeks. The post mortems on 19 of these chicks showed a hemorrhagic condition, the hemorrhage spots in a few cases being peritoneal clots but more frequently small blotches along the muscles of the femur and sternum. These chicks looked strong, healthy and vigorous until a few hours before death. The death of eight chicks in this group in the fourth week was the result of inserting the identification wing bands in the web of the wing which caused a fatal hemorrhage, bleeding continuing for from 12 to 48 hours before death.

GROUP XVII. *Cod Liver Meal:* At no time during the eight weeks duration of the experiment did the chicks in this group attain normal weight. The chicks always had an unthrifty appearance and evident lack of vigor but only 5 chicks were lost, these showing no one particular post mortem condition.

GROUP XVIII. *Gelatin:* The chicks in this group were always distinctly abnormal, their growth was slow, and their appearance sickly, the plumage being ruffled. The mortality was heavy and regular in frequency. No typical post mortem condition was observed.

GROUP XIX. *Casein, Marmite:* From the beginning these chicks grew excellently. They appeared normal in every respect at the conclusion of the eight weeks. No mortality occurred after the first week when one chick was lost.

GROUP XX. *Casein, Brewer's Yeast:* These chicks grew well for the first two weeks. During the third week, rapid growth continued with very little mortality, but the chicks exhibited a peculiar nervousness when the pen was approached. Six chicks died in the fourth and fifth week, all showing the same ante-mortem condition. The birds lay prone on one side with the legs outstretched and the head twisted back in a typical polyneuritic manner. Death followed in 12 to 24 hours. No gross pathological condition was observed on post mortem examination. After the fifth week, there were no further losses, the chicks continuing to grow normally. The ratio of units of food required to produce a unit of gain with the different groups was as follows:

Group	XIV.	Buttermilk powder	2.4:1
"	XV.	Meat meal	2.8:1
"	XVI.	Fish meal	2.4:1
"	XVII.	Cod-liver meal	3.5:1
"	XVIII.	Gelatin	3.8:1
"	XIX.	Casein—marmite	2.6:1
"	XX.	Casein—brewer's yeast	2.3:1

In Charts 5 and 6 are recorded the mortality and the average growth of these groups. It will be noted that only with cod liver meal as the protein

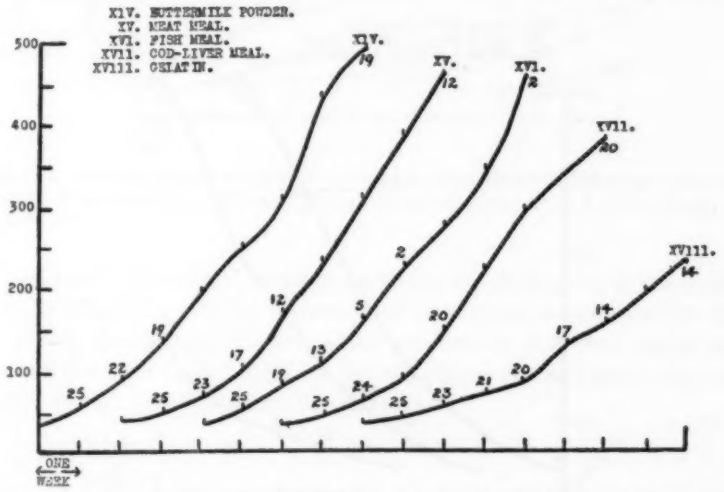

CHART 5.—Showing the growth with the different proteins when supplementing a basal diet composed of marmite 15%; cod-liver oil 3%; bone ash to 4.36% total ash, and white rice to 100.

supplement was growth subnormal. From the fifth week only two birds remained in the fish meal group; their growth, however, continued normally. Poor growth was obtained when the basal ration was supplemented by gelatin. The growth with the gelatin diet, however, was such, that the protein of marmite and the small amount of protein (2.6 per cent) from the white rice may have had a marked supplementary effect on the comparative growth, with the different protein concentrates. The heavy and characteristic mortality in Group XX as compared with Group XIX would suggest that 15 per cent of the dried brewer's yeast used in this and in previous experiments, was close to the minimum required to maintain normal nutrition. It would appear that the amount of vitamin G (B_2) was adequate, as good growth was attained by the surviving birds. The ante-mortem

symptoms exhibited by the chicks suggested that vitamin B_1 was deficient in the particular yeast used.

Fifth Series

It occurred to us that, since replacing the dried brewer's yeast in the diet by an equivalent amount of marmite reduced the high mortality to nil, the limiting factor in the synthetic basal diet used in our earlier experiments might still be the brewer's yeast. We desired to find some substitute for the large amount of white rice used in these diets as evidently the small

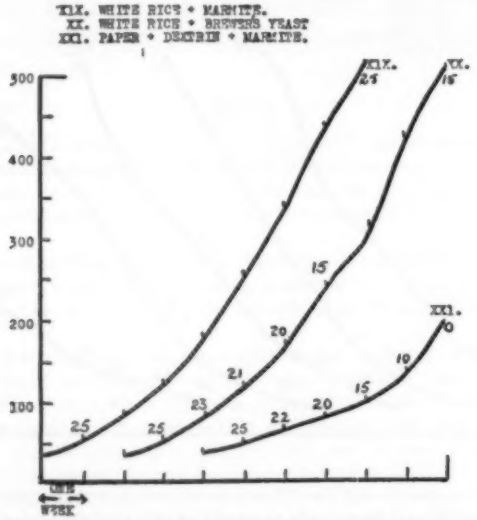

Chart 6.—Showing the comparative growth with casein as the source of protein in different simplified diets.

amount of protein in the rice was of good biological value. Our next attempt to obtain normal growth with a more strictly simplified diet was made by supplementing the following basal diet with an amount of each of the different fat-free protein concentrates sufficient to add 12.5 per cent crude protein to the diet, thus keeping the total protein the same as with the fourth series of experiments.

Marmite	15%
Cod-liver oil	3%
Paper pulp	3%
Bone ash to	4.36% of total ash.
Dextrin to	100.

In Chart 7 are shown the results in growth and mortality of this feeding trial. While the growth with fish meal and cod-liver meal was for some unexplained reason an improvement on the growth obtained with buttermilk powder or meat meal, in all cases the results were a failure, growth being

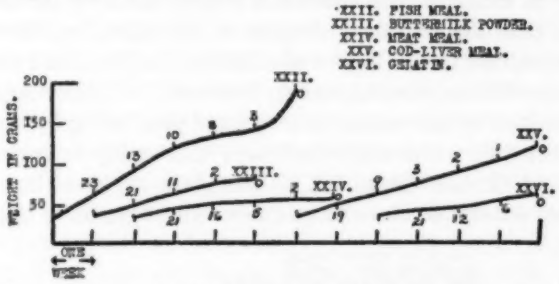

CHART 7.—Showing the growth with the different proteins when supplementing a basal diet composed of marmite 15%; cod-liver oil 3%; paper pulp 3%; bone ash to 4.36% total ash, and dextrin to 100.

decidedly sub-normal and no chick surviving the sixth week of the experiment. In Chart 8 are shown the results of an attempt to improve this diet by varying the amount of fibre. Three per cent of fibre from paper pulp gave the best results, but in all the groups the growth was again very poor and the mortality 100 per cent in six weeks.

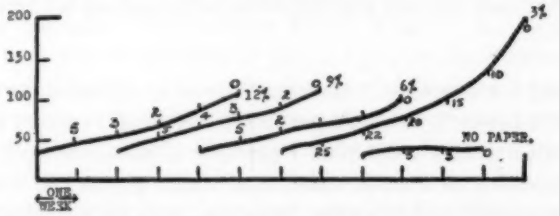

CHART 8.—Showing the effect on growth of varying the amount of fibre (paper pulp) in a diet composed of 12.6% protein from casein; marmite 15%; cod-liver oil 3%; bone ash to 4.36% total ash, and dextrin to 100.

That the occurrence of leg-weakness among chicks reared in confinement on a synthetic diet could be prevented by increasing the amount of dried brewer's yeast to 40 per cent of the diet, has already been discussed. We have found, however, that keeping the amount of brewer's yeast constant at 15 per cent and changing the composition of the remainder of the basal synthetic diet, *i.e.*, replacing the paper and dextrin by white rice made the diet entirely adequate for normal nutrition. It occurred to us that possibly

the small amount of vegetable protein in the white rice was the cause of marked improvement and that it was not possible to grow normal chicks on a diet in which the protein was entirely of animal origin. It seemed possible that, as the large amount of yeast required to prevent leg-weakness was greatly in excess of the vitamin B requirements of the chick, some factor other than the vitamin B complex of the yeast, *i.e.*, the yeast protein, was responsible for the improved nutrition. In Chart 9 are recorded the results of a preliminary experiment to determine whether normal growth could be obtained by increasing the amount of yeast using 5 per cent of the residue of brewer's yeast which had been thoroughly extracted with 90 per cent ethyl alcohol. Group XXXI was given the same basal diet but with an equal amount of crude protein from wheat gluten to that added in

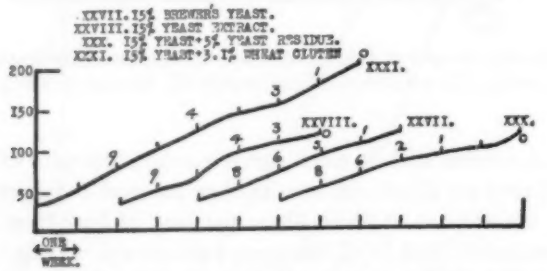

CHART 9.—Showing the effect on growth of different additions to a diet composed of 18% protein (buttermilk powder); 10% paper pulp; 15% cod-liver oil; fish meal ash to 8.9% total ash, and dextrin to 100.

the 5 per cent yeast residue. There was little or no difference in the results obtained with brewer's yeast, 90 per cent ethyl alcohol extract of brewer's yeast or with brewer's yeast plus 5 per cent alcohol extracted yeast residue. The addition of a small amount of wheat gluten did considerably improve the growth. The results, however, were uniformally poor, the total protein of the diets being probably too high.

DISCUSSION

Our experiments using synthetic diets have not been so satisfactory as we had hoped. We have encountered many difficulties which show definitely that the nutritional requirements of the chick cannot be adequately met by the synthetic diets being used successfully with rats. Our original objective to study the comparative value in avian nutrition of different proteins when fed as the sole source of protein in an otherwise synthetic diet, has not been attained.

It has been found necessary, if normal growth is to be obtained, to include in the basal diet food stuffs such as marmite and white rice or brewer's yeast and white rice, which add to the diet a considerable amount of vegetable protein. A diet composed of casein 12.2 per cent, marmite 15 per cent; cod-liver oil 3 per cent; salt mixture 4.4 per cent and white rice 61.8 per cent *i.e.*, Group XIX, grew perfectly normal chicks with no mortalities after the 1st week of growth but when 3 per cent paper pulp and dextrin was ground into the diet in place of the white rice and the total protein of the diet was kept the same by increasing the casein to 15.8 per cent *i.e.*, Group XXI, more or less complete failure resulted, none of the 25 chicks surviving the sixth week of the experiment. The poor growth could not be attributed to the fibre content of the diet as varying the amount of paper pulp (see Chart 8) did not result in any improvement. The difference between success and failure with Group XIX and Group XX can be due only to some substance present in the white rice. While Susuki (1927) has shown the protein of white rice to be of good biological value it would be very surprising that the small amount of rice protein 2.6 per cent added to these diets should have such a pronounced supplementary effect on casein and more particularly on the protein of buttermilk powder, Group XIV, Chart 5. However, the improved growth resulting from the addition of a small amount of wheat gluten, 3.1 per cent to a synthetic diet (Group XXXI, Chart 9) would suggest that the effect of the inclusion of white rice in the diet might be due to the rice protein. Comparing the results with Group XXVI (Chart 8) and Group XVIII (Chart 5) there is no doubt that 2.6 per cent protein from white rice had a marked supplementary effect on the protein of marmite and gelatin. However, it can only be concluded from these experiments that the addition of white rice to these synthetic diets, proven to contain adequate amounts of the known vitamins essential for growth in the chick, had a pronounced effect in improving the growth and reducing the mortality, which cannot be explained from our present knowledge of the nutritional requirements of the fowl. That the effect is due to the rice protein, and that some protein of vegetable origin, or some unknown factor associated with vegetable material, is necessary for normal nutrition of the chick is suggested, but has still to be definitely proven by further experiments.

Equal amounts of crude protein from buttermilk powder, fish meal or meat meal had practically the same effect on growth when supplementing a basal diet composed of marmite 15 per cent; cod-liver oil 3 per cent; bone ash to 4.36 per cent total ash and white rice to 100. However, supplementing the same basal diet with cod-liver meal gave sub-normal growth

indicating the protein of this material to be of poorer biological value so far as growth is concerned. The amount of food required per unit of gain in body weight was much the greater in the cod-liver meal group and was comparable with that of gelatin. The mortality in the meat meal and fish meal groups was much greater than that with cod-liver meal and especially buttermilk powder. The losses from hemorrhage were confined to the meat meal and fish meal groups and particularly to the latter. A later paper will discuss this condition showing that with the same basal diet but with the fish meal unextracted by ether, no chicks were lost from hemorrhage and normal growth was obtained. Taking the mortality into consideration, the best results were obtained with buttermilk powder or casein.

There would appear to be little or no doubt that the leg-weakness which occurred with Groups I, VI and VII was in no way associated with the vitamin D content of the diet. Increasing the amount of medicinal cod-liver oil from 2 to 5 per cent had no effect nor was increased exposure to ultra-violet light beneficial. The bone ash and calcium and phosphorus content of the blood serum was quite normal. Varying the ratio of protein to yeast vitamin B within reasonable limits (Group XIII) had no marked effect on the condition, nor was the fibre content of the diet found to be associated with the incidence of this particular form of leg-weakness. The only significant difference between the diet with Group VI which developed extreme leg-weakness and the diet with Group XIV which grew normally was the substitution in the latter diet of white rice for paper pulp and dextrin.

Summary

1. Equal amounts of crude protein (N×6.25) from buttermilk powder, fish meal or meat meal had practically the same effect on growth when supplementing a basal diet composed of marmite 15 per cent, cod-liver oil 3 per cent, bone ash to make the total ash of all the diets 4.36 per cent, and white rice to 100. Taking into account the high mortality with the fish meal and meat meal diets, the best results were obtained with buttermilk powder. Supplementing the same basal diet with an equal amount of crude protein from cod-liver meal gave subnormal growth, indicating the protein of this material to be of poorer biological value so far as growth is concerned. In all cases the protein supplements were freed from the variable amount of fat which they contained by extracting with ether.

2. An attempt to reduce the amount of extraneous nitrogenous substances in the basal diet by substituting ground paper pulp and dextrin for the white rice resulted in more or less complete failure in growth. Vary-

ing the amount of fibre (paper pulp) in the diet did not result in any significant improvement.

3. It has not been found possible to grow normal chicks on a diet in which the sole source of protein is of animal origin. The inclusion of white rice in the simplified basal diets used in these experiments had a pronounced effect in improving the growth, preventing the occurrence of leg-weakness, and reducing the mortality, which does not appear to be explainable from our present knowledge of the nutritional requirements of the growing chick. Some evidence is discussed which suggests that the effect is due to the rice protein and that some protein of vegetable origin, or some as yet unidentified substance, or substances, associated with vegetable material is necessary for normal chick nutrition.

Our thanks are due to Professor W. R. Graham and to Professor H. L. Fulmer for their interest and help throughout this work, the expenses of which were in part defrayed by a grant from the British Empire Marketing Board. We are also indebted to Mr. W. A. Munn, St. John's, Nfld., for supplying the medicinal cod-liver oil and the cod-liver meal used throughout these experiments.

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THE EFFECTS OF RADIANT ENERGY ON EXPERIMENTAL HEMOLYTIC ANEMIA*

By

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PREVIOUS observations (Laurens, 1928; Mayerson and Laurens, 1928), demonstrating that repeated irradiation of normal animals with the flaming carbon arc stimulates the hematopoietic system to a sustained production of a greater than usual number of red cells, encouraged the belief that such radiation might be particularly effective in stimulating blood regeneration in anemia. In a recent paper we (Laurens and Mayerson, 1931) report the results of irradiating 24 dogs made anemic by hemorrhage, and show that irradiation with the flaming carbon arc or the quartz mercury lamp produces marked and persistent increases in the number of erythrocytes and reticulocytes, particularly following long exposures, but fails to influence the regeneration of hemoglobin. Such experiments are a very severe test of the efficiency of radiation. Not only is hemoglobin removed from the body, but the diet used furnishes only a bare minimum of material which could be used in the manufacture of new hemoglobin and radiant energy cannot act as a substitute for dietary deficiencies. An hemolytic anemia affords a more favorable condition for radiation studies for there is but little loss of hemoglobin from the body and the retained hemoglobin may be utilized in the formation and maturation of new and functional red blood cells.

This report deals with the effects of radiation on dogs made anemic by the injection of acetylphenylhydrazine (pyrodin), which is just as effective in blood destruction as is phenylhydrazine hydrochloride but much less toxic to the liver (Bodansky, 1923; Long, 1926). The literature dealing with the experimental and clinical use of phenylhydrazine and its derivatives has been reviewed by Brown and Giffin (1926), Long (1926) and Giffin and Allen (1928). These compounds are strong reducing agents and split the hemoglobin into its pigment and protein fractions. The basic drug becomes oxidized, setting free a benzol ring which is the active agent in bringing about the blood destruction.

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The experimental procedures and methods have been given in earlier papers. The animals when received were bathed, purged and placed in large, roomy cages and fed the standard salmon bread diet of Whipple and Robscheit-Robbins to which additional salmon was added at times as an appetizer. Determinations were made of red and white blood cells, reticulocytes, hemoglobin, hematocrit, platelets, red cell fragility, specific gravity and total blood volume. When constant values had been reached, subcutaneous injections of various amounts of acetylphenylhydrazine were given. The original intention was to inject an amount of the drug sufficient to produce a severe anemia and to maintain the anemia at this level by further small injections. The development of a tolerance to the drug, however, made this method extremely difficult. Thus, the administration of 217 mg. (60 mg. per 100 cc. red cells) of pyrodin to Dog 8 produced in 7 days levels of 57 and 54 per cent of the normal for the red blood cells and hemoglobin respectively. A similar injection (212 mg., 60 mg. per 100 cc. red cells) one month later was followed by much smaller changes, the maximum low levels of 82 and 71 per cent normal in red cell count and hemoglobin, respectively, being reached in 19 days. A third injection, 6 weeks later, of 211 mg. (60 mg. per 100 cc. red cells) again produced only a similar slight grade of anemia (red cells = 79 per cent, Hb. 82 per cent normal) but in 22 days. Clinicians likewise report that a reduction dose may become a maintenance dose.

The dosage was calculated according to the total number of circulating red cells, the amounts injected varying from 40 to 100 mg. per 100 cc. of red blood cells (total amounts equal to from 104 to 876 mg.). In two experiments the dose was administered in two parts with a two day interval between the injections, in two others the dose was given in three successive injections. Single injections were employed in the remaining experiments. Determinations were made throughout the development of the anemia, and until pre-injection levels had been approached or reached. In several experiments the recovery proceeded to higher levels than before injection. With only one exception, the animals reacted favorably even when large amounts of the drug were injected. The one exception showed a very severe anemia (hemoglobin 15 per cent normal), and the animal was very weak and required careful attention for several days.

A total of twenty-four animals was used, five being exposed to the radiant energy emitted by a Cooper-Hewitt air cooled quartz mercury vapor lamp, eight to that emitted by a Pan-Ray flaming carbon arc burning "Sunshine" carbons, and the remainder serving as controls. The carbon arc, operated at 24 to 28 A and 50 to 60 V, emits an average of 0.820 gm.-

cal. per sq. cm. per minute of energy incident at 1 M. and has a distribution of 5 to 6 per cent in the ultra violet, 29 to 31 per cent in the luminous and 63 to 66 per cent in the infra red. The quartz mercury vapor lamp, operated at 4 to 5 A and 70 to 80 V, emits an average total energy of 0.0619 gm.-cal. per sq. cm. per min. incident at 1 M. with a distribution of 13 per cent in the ultra violet, 7 per cent in the luminous (400–1400 mμ) and 80 per cent in the infra red. In each case the abdomen and thorax were exposed. Various modifications of the period and time of exposure were used, the irradiation being started before the injection of the acetylphenylhydrazine, immediately after, or at the time at which the anemia was most pronounced. The exposures in the quartz mercury vapor arc series ranged from 15 minutes at 80 cm. (1.46 gm.-cal. per sq. cm.) to 30 minutes at 70 cm. (3.78 gm.-cal. per sq. cm); in the carbon arc series from 15 minutes at 80 cm. (19.2 gm.-cal. per sq. cm.) to 60 minutes at the same distance (76.8 gm.-cal. per sq. cm).

Twenty-four hours after the injection of acetylphenylhydrazine there was always some degree of anemia and this ran essentially the same course as illustrated by the figure. Under no conditions did the radiation have any apparent effect on the severity or rate of development of the anemia. The destruction of red cells after the injection of pyrodin was rapid for the first two or three days, and was most marked at the end of from five to nine days, the red blood cells and hemoglobin levels at this time being between 15 and 82 per cent of the original levels. The mean low levels in the twenty-four animals was 50 per cent, and the average period for the attainment of this level 8 days. The rate of the development of the anemia is slower when the dose is divided but single injections produce the same amount of destruction. The degree of anemia is in large measure regulated by the resistance of the cells of the particular animal or person to the drug and the regenerative activity of its hematopoietic system, rather than by the amount of drug injected. Thus the injection of equivalent amounts of pyrodin (277 and 297 mg. per 100 cc. red cells) into two animals of about the same weight (13.6 and 14.6 K.) produced a maximal anemia in 9 days in the first dog, the red blood cell count at this time being 49 per cent normal, while the second animal developed only a much milder anemia which reached its peak at the end of three days, the red blood cell count being 81 per cent normal at this time. The average number of red blood cells per cu. mm. of blood destroyed per day per mg. of the drug varies from 350 to 2000, the average of all the animals being 1200.

The sharp drop in the red blood cells during the first few days and the subsequent gradual decrease in their destruction suggests a strong and im-

mediate stimulation for the liberation of new cells. The end point of the anemia is reached when this production balances destruction. This stimulus to regeneration and liberation is indicated by the marked increase in the number of reticulocytes. The normal percentage varied from 0 to 3 per cent of the red blood count. Two or three days after the injection of pyrodin, this was increased in most cases to almost double, the count re-

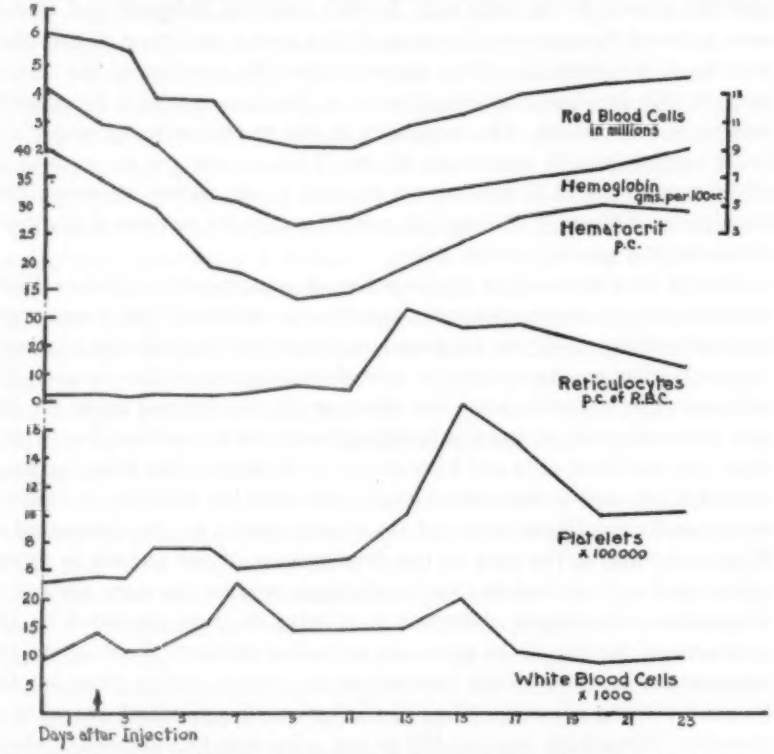

FIG. 1. Changes in blood cytology following injection of acetylphenylhydrazine (pyrodin). Dog. 4. Two subcutaneous injections of 233 mgs. each (40 mgs. per 100 cc. red blood cells); arrow indicates time of second injection.

maining at this level or increasing slightly. The first indication of recovery from the anemia was usually preceded by another sudden and marked rise. In ten experiments the reticulocytes were over 10 per cent of the red blood count at this time, the value reaching 33 per cent in one experiment. Further evidence of regenerative stimulation was shown by the marked spurts in platelets which occur along with the similar changes in the

reticulocytes. These platelet changes are transitory, the count soon returning to normal levels except in those cases in which the regeneration is particularly active. Thus in experiment 4, where the average reticulocyte and platelet counts before the injection were 3 per cent and 582,000 per cu. mm. respectively, there was a slight rise in both counts during the development of the anemia, followed by sharp supplementary increases to 33 per cent in the reticulocytes and 1,000,000 per cu. mm. in the platelets on the thirteenth day after the injection, at which time the red cell count was beginning to rise. The reticulocyte level gradually decreased as the number of reds increased, so that 10 days later the value was 13 per cent of the red count. The platelet level also showed a sharp drop but was still high at the end of the period of observation.

The curve of hemoglobin destruction usually parallels that of the red blood cells quite closely. Corpuscular hemoglobin, obtained by dividing the hemoglobin per 100 cc. of blood by the number of cells per cu. mm., is used as an index of the amount of hemoglobin in each cell. Corpuscular volume, calculated by dividing the volume of cells per 100 cc. by the number of cells per cu. mm. of blood, denotes the average volume of the individual cell. The corpuscular saturation, or actual proportion of the substance of each cell taken up by hemoglobin, is obtained by dividing the corpuscular hemoglobin by the corpuscular volume or directly by dividing the hemoglobin content expressed in gm. per 100 cc. by the hematocrit value expressed in cubic centimeters per 100 cc. Corpuscular volume usually showed marked increases and in twelve experiments the anemic level was 20 to 50 per cent greater than before the administration of the drug. This increase in the volume of the individual cells begins almost immediately after injection and persists throughout the recovery period. Values ranging from 70 to 95 cu. μ were frequently encountered, as compared with the average value of 59.3 cu. μ determined for 60 normal animals (Mayerson, 1930). During the development of the anemia, the corpuscular hemoglobin showed only minor fluctuations from pre-injection values, an indication that the hemoglobin liberated from the cells is not immediately available. This results in a lowered corpuscular saturation. In many of the experiments, the cells were only about 80 per cent saturated, while in some, values as low as 50 and 60 per cent saturation were obtained.

The destruction of the red cells and of the hemoglobin was accompanied by a rise in the icterus index, which, however, was not so great as would have been expected in view of the fact that the clinical use of phenylhydrazine often leads to jaundice. Determinations of serum bilirubin were negative in all cases, as Long (1926) reported in rabbits after pyrodin in-

jection, which indicates a low threshold and very rapid disappearance of the breakdown products of hemoglobin from the blood of the dog. During the first week after the injection of the drug, and particularly for the first few days after, the blood in many cases was decidedly darker in color than normally. During this period the hemoglobin calculated from oxygen capacity gave values lower than those obtained with the calibrated hemoglobinometer. Determinations by the Stadie method indicated the presence of some pigment other than hemoglobin, but spectroscopic examination for methemoglobin was negative, even when the blood was decidedly dark brown.

As the anemia developed, the resistance of the cells to hypotonic solutions of sodium oxalate increased, reaching a maximum either just before or at the point of lowest blood count and returning to normal during the recovery period. Various explanations have been proposed for this change, increased cholesterol content of the corpuscles, and increased amount of stroma among others. Perhaps the presence of large numbers of reticulocytes, which many believe to be quite resistant, is a contributing factor. Our experiments do not support the contention that the increased resistance of the red blood cells explains the acquisition of the tolerance to phenylhydrazine. In the three cases in which injections were repeated at intervals of 4 to 6 weeks, the fragility of the cells returned to normal before each subsequent injection, but the animals nevertheless showed a decided tolerance to the drug. It is possible that these cells may have been more resistant to pyrodin and not to sodium oxalate since Long (1926) was able to show changes in the resistance of rabbit cells to phenylhydrazine solutions but not to sodium chloride.

The changes in the leucocytes were irregular. In four experiments there was no change in the levels that could not be ascribed to normal variations. In nine cases there was a sharp rise (in one case to 33,000) following the injection which persisted for 1 or 2 days and was followed by a return to normal levels. In two of these experiments there was a secondary rise at the point where the red blood cell count was lowest. In the remaining nine experiments, the leucocyte count increased in inverse proportion to the red cell count, the highest counts (50 to 200 per cent normal) being found at the point of greatest blood destruction, followed by a return to normal. In two of these experiments the count remained high throughout the recovery period. Giffin and Allen (1928) also found an irregular response to the injection of phenylhydrazine HCl in dogs, and raised the question as to whether the leucocytosis was secondary, a result of tissue destruction, or primary, due to stimulation of bone marrow. Our experiments show both

types of responses. The first group, showing a sharp rise and decrease after the drug was injected, indicates a specific stimulus. Two of these animals, however, showed a secondary rise at the anemia level, which would suggest that this leucocytosis was due to secondary stimulation as a result of the destruction of red cell substance. The second group illustrates secondary stimulation, the increase in the number of whites being consequent to red cell destruction and proportional to it. The leucocytosis is generally due to increase in the number of the neutrophiles at the expense of the lymphocytes. There was no definite shift in the distribution in 6 cases, while 3 showed slight increase in lymphocytes and proportional decreases in neutrophiles. Normoblasts and transitional forms were common and in the severe anemias quite numerous, one animal (Dog 14), for example, having a transitional count of 1375 for two days. These forms disappear as soon as recovery begins, suggesting that the minimal level of the red cells was in part the result of bone marrow injury. Mononuclears and eosinophiles showed little change, and basophiles were seldom seen.

Although the radiant energy did not exert any definite influence on the development of the anemia, it markedly affected the rate and extent of recovery therefrom. The table illustrates the regeneration in sixteen animals, eight irradiated and eight controls, which developed corresponding degrees of anemia. The values are given in per cent of normal, a value representing the average of at least three separate sets of determinations made over a period of at least a week. For the sake of brevity and simplification, the table includes only the lowest value reached and the succeeding values at five day intervals.

The average anemia levels were the same for the two groups. There was somewhat more extensive hemoglobin than red cell destruction resulting in a corpuscular hemoglobin smaller than normal. Furthermore the volume of the individual cells increased and thus the corpuscular saturation was also considerably below normal in both groups. Five days later, the red cell number in both groups showed approximately the same amount of variation and increase. The hemoglobin regeneration was definitely better in the irradiated group, with an average gain of 17 per cent as compared with a 10 per cent gain in the controls. This difference became more marked as regeneration proceeded. At the end of ten days, the average red blood cell count in the controls had increased 20 per cent, while the irradiated group showed a gain of 30 per cent over the low anemic value. Similarly, the average hemoglobin value of the controls at this time was 25 per cent above the anemic level while the average value for the irradiated animals was 34 per cent above the low level, seven of the latter group showing values of

from 30 to 44 per cent higher. This difference in regeneration persisted. Fifteen days after the lowest level of the anemia the blood counts of the irradiated animals were from 80 to 96 per cent of the normal (except Dogs 9 and 12 which were only 72 per cent normal) as contrasted with those of the control group which were from 67 to 83 per cent normal, only two, however, showing values of 75 and 83 per cent respectively. The average hemo-

COMPARISON OF REGENERATION OF CONTROL

Dog No.	Wt. Kg.		Red Blood Cells Per cent normal				Hemoglobin Per cent normal				Hematocrit Per cent normal			
			Lowest Value	Days After Low Value 5	10	15	Lowest Value	Days After Low Value 5	10	15	Lowest Value	Days After Low Value 5	10	15
8	11.8	Controls	57	62	63	67	54	50	59	73	65	73	81	87
4	10.0	"	33	63	74		27	42	58	69	33	47	70	71
17	13.6	"	49	70	71	83	39	69	86	98	60	67	80	90
13	7.0	"	57	56	67	68	60	61	70	79	86	70	80	90
7	9.0	"	38	40	66	70	47	50	79		57	57	97	90
2	6.4	"	46	54	67	68	45	70	88	79	44	80	111	107
28	18.6	"	60	65	72	75	65	69	75	77	83	84	79	97
1	6.2	"	60	70	78	70	44	50	69	44	44	59	81	62
		Average	50	60	70	72	48	58	73	77	59	67	85	87
9	9.0	C Arc	54	80	98	72	68	81	88	90	77	108	114	108
12	9.0	"	35	50	64	72	25	53	59	70	47	47	61	80
19	10.6	"	44		88	86	41		85	93	52	92	97	97
24	8.6	"	52	61	76	92	44	57	74	80	63	69	93	91
22	12.0	Hg Arc	38	44	60	80	32	53	70	71	43	59	79	79
27	13.2	"	46	50	75	93	48	58	80	85	53	64	80	97
26	6.8	"	65	78	90	95	53	70	96	107	59	65	105	105
21	9.0	"	65	65	90	96	55	73	87	93	47	79		
		Average	50	61	80	87	46	63	80	86	55	73	90	94

globin level for the control group at this time was falsely high because of the unusual regeneration in one dog, No. 17, the values for the remaining animals being between 44 and 79 per cent normal. Two animals in the irradiated group (12, 22) showed similar low levels, the values in the others being 80 to 107 per cent normal.

The corpuscular volume remained greater than normal in both groups, but was more so in the control than in the irradiated animals. A probable explanation of this is that the newer cells appearing in the circulation of the latter as a result of the radiation were smaller and since regeneration was

more active in these animals the average corpuscular volume is lower than in the control group. The production of small cells following irradiation has been demonstrated in our previous studies. The fluctuations in corpuscular volume were similar in both groups, but the cells of the irradiated animals of smaller volume were more saturated than were those of the controls. The last two columns of the table show the average daily gains in red

AND IRRADIATED DOGS

Corpuscular Volume Per cent normal				Corpuscular Hb Per cent normal				Corpuscular Saturation Per cent normal				R.B.C. average daily gain for 15 days	Hb average daily gain for 15 days gms.
Lowest Value	Days After Low Value			Lowest Value	Days After Low Value			Lowest Value	Days After Low Value				
	5	10	15		5	10	15		5	10	15		
113	120	129	128	89	89	88	110	83	80	72	85	73,333	0.18
101	100	109	96	81	91	81	80	80	92	94	83	167,333	0.35
126	105	120	114	83	99	123	119	65	102	100	110	158,667	0.42
140	107	116	120	97	96	98	103	66	85	84	84	78,000	0.16
152	145	146	128	123	126	119		80	108	80	100	134,667	0.26
91	149	171	160	107	123	124	108	131	95	85	79	82,667	0.20
136	130	107	128	106	105	83	101	79	81	78	79	73,333	0.11
75	80	104	87	74	74	89	90	98	85	85	103	46,667	0.15
117	117	125	120	95	100	101	102	84	91	85	90	101,833	0.23
143	134	116	130	126	100	90	93	88	74	80	73	130,667	0.11
137	96	105	96	73	107	100	105	50	111	95	109	145,333	0.32
121	135	110	108	82	85	86	100	69	64	86	93	160,000	0.46
121	122	122	95	83	102	96	83	66	84	79	88	146,667	0.24
110	133	131	121	86	121	109	109	62	90	84	90	153,333	0.34
123	137	109	111	110	121	113	98	89	88	104	86	200,000	0.40
90	83	117	110	83	91	102	112	90	109	87	103	100,000	0.41
73	81			72	105	91		111	109	147	115	100,000	0.22
115	115	116	110	89	104	98	100	78	91	95	95	142,000	0.31

blood cells and hemoglobin for the fifteen days following the lowest anemic levels. Not only were the averages in the irradiated group greater than were those of the controls but the individual values were distinctly higher. Only three control animals show an average daily gain of over 100,000 red blood cells, which is the lowest gain recorded in the irradiated group. Similar differences are apparent in the regeneration of hemoglobin. Specific gravity and plasma volume determinations show that these changes are real and not due to dilution or concentration of the blood. No significant

differences could be detected in the behavior of the reticulocytes, white cells or the resistance of red cells in the control and irradiated groups.

Our failure to obtain active regeneration of hemoglobin in our previous studies on hemorrhagic anemia and our success in these experiments on hemolytic anemia are due to the difference in the experimental conditions. A number of workers report that blood regeneration proceeds more vigorously in anemias caused by phenylhydrazine than in anemias due to hemorrhage in which the blood is actually lost. Injection of the lost blood has been claimed to remove this difference, the products of red cell destruction supposedly stimulating formation. Whipple and Robscheit-Robbins demonstrated that a very large portion of hemoglobin introduced either intravenously or intraperitoneally into the severely anemic dog may be conserved for the building of new and much needed hemoglobin. They further noticed that in most instances there occurs a certain lag in the output of hemoglobin following parenteral introduction of the blood pigment. One may believe that the hemoglobin is tucked away somewhere, or one may choose to assume that the hemoglobin is first broken down to end or intermediate products which are stored and used exactly as though these substances came from the digestive tract. Evidence seems to favor the latter view.

In a hemolytic anemia there is no actual loss of blood but instead a large store of liberated hemoglobin is made available. It hardly seems plausible that the hemoglobin would be utilized as such directly; but rather that it is broken down and worked over in some fashion or other for the rebuilding of new cell structures and hemoglobin. This process may take some time and this may perhaps be an additional cause for delay before regeneration can begin. In spite of this delay the final recovery is complete at an earlier date than that following hemorrhagic anemia. Itami and Ritz indicate that repair from phenylhydrazine injury is more rapid than that following loss of blood by actual removal. Price-Jones offers a very apt criticism of their experiments, since their conclusions were based on red cell and hemoglobin values and not on any estimate of total circulating hemoglobin (Robscheit-Robbins, 1929). Krafka (1930) also finds little or no difference in the rate of regeneration following hemolysis and hemorrhage, although Johnson and Berglund (1928) report an exceedingly rapid regeneration of the erythrocytes in phenylhydrazine anemia, with reticulocyte counts as high as 41.3 per cent.

The question as to the mode of action of radiation in speeding up regeneration is a difficult one to answer. The radiation certainly does not exert any marked detoxicating action on the drug after injection, for there is

little difference in the grade of anemia or in the time necessary for its development in the irradiated and control animals. This also rules out any greater secondary stimulation due to greater destruction. The cells in both groups show no significant differences in their resistance to hypotonic solutions of sodium oxalate. The effects of the radiation are on the recovery process after the most marked destruction is over. The experiments of Osato and Tanaka (1929) are interesting in this connection. They studied the effect of radiation on regeneration and iron metabolism in dogs, guinea pigs, and rats and state that the blood iron increased at the expense of the liver and bone marrow iron. The feeding of an iron-containing product,[1] resulted in deposits of iron in the spleen and liver which disappeared following irradiation along with marked blood regeneration. The authors conclude that following irradiation the reserve iron is withdrawn from the organs and is used in the manufacture of hemoglobin. The better regeneration following irradiation noted in our experiments may be due to a more efficient utilization of the iron made available by the destruction of hemoglobin. The action of radiation on the pigments set free may also be an important factor.

Summary

Anemia was produced in twenty-four adult dogs on a standard diet by the subcutaneous injection of acetylphenylhydrazine (pyrodin), the dosage being calculated in terms of red cell volume and varying from 40 to 100 mg. per 100 cc. of red cells. Eight animals were irradiated with the flaming carbon arc burning "Sunshine" carbons, five with the quartz mercury lamp and the remainder served as controls.

No significant differences in the rate of development or in the severity of the anemia were observed in the irradiated and non-irradiated animals. Some degree of anemia, coincident with an increase in the icteric index, was present in all cases at the end of 24 hours. The anemia became most marked in from 5 to 9 days, the red cell and hemoglobin determinations at this time showing values of from 15 to 82 per cent of the original levels. Single injections produced a similar degree but a more rapid development of the anemia than did divided doses of the same amount. Marked increases in reticulocytes and platelets occurred, and, in many cases, these constituents showed a further secondary rise at the beginning of regeneration. The corpuscular volume increased and remained high. The resistance of the cells to hypotonic sodium oxalate increased gradually and was greatest at the height of the anemia.

[1] Blutose.

The regeneration of the red cells and hemoglobin was unquestionably faster in the irradiated group, the carbon and mercury arcs being equally effective. The average cell and hemoglobin values of eight animals of the control group 23 days after the injection and 15 days after the lowest anemic level were 72 and 77 per cent normal respectively. The average values for the same number of irradiated animals that developed a comparable degree of anemia were 87 and 86 per cent normal. No significant differences in reticulocytes, whites, platelets and red cell fragility were observed.

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THE PHYSIOLOGICAL EFFECT OF RATIONS RESTRICTED PRINCIPALLY OR SOLELY TO THE ALFALFA PLANT

II. CYSTINE AS A LIMITING FACTOR IN THE NUTRITIVE VALUE OF ALFALFA PROTEINS*

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ALFALFA is grown so abundantly and cheaply in extensive regions of the West that the relatively high price of concentrates has led to the frequent practice of restricting dairy cattle largely to alfalfa. This practice and the fact that such cattle have at times (1) been found to be in negative nitrogen equilibrium, suggested the desirability of obtaining more definite information than we now possess on the biological value of alfalfa proteins. The extensive use of alfalfa as a consitituent of more or less well balanced rations has amply demonstrated its excellent feeding value but has not yielded specific information on the adequacy of rations in which alfalfa furnishes the principal or sole source of proteins.

The more limited number of experiments in which alfalfa furnishes the principal or sole source of nitrogen may be interpreted as indicating that alfalfa crude proteins possess a nutritive value that places them in about the same class as those of the corn kernel. Hart *et al* (2, 3) interested in determining the nutritive value of so-called non-protein nitrogen, concluded that the nitrogen of alfalfa hay possesses approximately the same efficiency for growth and milk production as that of the corn grain. Nevens (4) obtained a biological value of 62 for alfalfa proteins, when fed to rats at a 9 per cent level of intake. Just recently Sotola (5) reported a biological value of 56 for alfalfa hay proteins when fed to lambs at a level of protein intake equivalent to approximately 14 per cent of the dry matter consumed. Such data would seem to indicate that attention can profitably be given to a study of the factors limiting the nutritive value of the crude protein of alfalfa.

Several years ago (6) the writer obtained evidence indicating that the

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nutritive value of the crude protein of alfalfa hay is limited by its cystine content. In view of the difficulties incident to feeding rats such a bulky material as alfalfa hay, from which it has not been found practical to extract the proteins, we have fed products designated as "alfalfa leaves." The 1927, 1928 and 1930 products were obtained from second cutting alfalfa hay which had been cut when in about 1/4 bloom. The 1929 product was obtained from second growth alfalfa which had been cut when in the early bud stage. These products, hereafter referred to as "alfalfa leaves," contained varying amounts of buds, blossoms and stems.

Our general procedure has been to feed otherwise supposedly satisfactory rations containing enough finely ground alfalfa leaves to supply from approximately 9 to 15 per cent of alfalfa crude protein. The experiments described in this paper are of two types. First, a series of group experiments in which rats were most commonly fed in groups of 3, the food intake recorded for the group, and the individual body weights recorded weekly. Second, a series of paired-feeding experiments, as employed by Mitchell and Beadles (7). The group experiments will be described first.

Group Experiments

It was soon found that rations containing alfalfa leaves as a source of crude protein, fed *ad libitum*, produced very little growth. Of 21 rats fed *ad libitum* on rations 47, 47a, 57 or 57a (See Table I) for periods varying from 6 to 15 weeks, the maximum gain made by any one rat was 25 gm. over a period of 14 weeks. This rat was one of a group of 5, whose consumption of ration 47 averaged 44 gm. per rat per week. The average weekly gain per rat for the entire group of 21 rats was $0.32 \pm .13$ gm. The average weekly food intake per rat for the various groups varied from 31 gm. for a group of small (approximately 40 gm.) rats to 51 gm. for a group of approximately 70 gm. rats. The average weekly intakes for the various groups were 31, 44, 44, 44, 50 and 51 gm., respectively.

In order to assess the significance of the food intakes encountered, and to determine the suitability of the types of rations fed, various groups of rats were restricted to various levels of rations 51 and 54 or fed *ad libitum* on rations 51 and 59. The groups to which rations 51 and 59 were fed *ad libitum* may be dismissed with the statement that over periods of 6 to 8 weeks, with food intakes averaging 85 gm. per week, an average weekly gain of 19.8 gm. per rat was obtained. Three rats restricted to an average of 40 gm. per week of ration 51 made an average weekly gain of 8.6 gm. over a period of 9 weeks. Three rats restricted to an average of 40 gm. per week of ration 54 made an average weekly gain of 3.6 gm. over a period of 9 weeks.

TABLE I

COMPOSITION OF RATIONS IN PERCENTAGE BY WEIGHT

	Crude protein (N×6.25)	Ration number									
		44	47	47a	51	57	57a	59	64	67	70
1927 alfalfa	24.06	50.00	62.5	—	62.5	—	—	—	—	—	—
1928 alfalfa	19.38	—	—	62.5	—	44.0	—	—	—	—	—
1929 alfalfa	27.40	—	—	—	—	—	44.0	44.0	—	35.5	40.0
1929 alfalfa	30.00	—	—	—	—	—	—	—	40.	—	—
1930 alfalfa	23.06	—	—	—	—	—	—	—	—	—	**
NaCl	—	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
$NaH_2PO_4 \cdot H_2O$	—	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	2.0
Na_2SO_4	—	—	—	—	—	—	—	—	—	—	.3
Starch	—	27.5	10.0	10.0	0.7	28.5	28.5	19.2	26.0	27.0	25.7
Sucrose	—	5.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0	15.0	10.0
Salad oil	—	10.0	15.0	15.0	15.0	15.0	15.0	15.0	—	—	—
Butter fat	—	4.0	4.0	4.0	4.0	4.0	4.0	4.0	19.0	19.0	19.0
Cod liver oil	—	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Dried yeast	47.34	—	—	—	—	—	—	—	1.5	—	1.0
Crude casein	78.48	—	—	—	9.0	—	—	9.0	—	—	—
l—cystine*	72.88	—	—	—	.3	—	—	.3	—	—	—
Total protein	—	12.03	15.04	12.11	22.32	8.53	12.06	19.34	12.71	9.73	11.43

* The cystine used in ration 46 was not tested for optical activity; most of that used in subsequent rations showed values for $[\alpha]_D^{25}$ varying from approximately −212° to −217°, as determined on 1% solutions in (N/I) HCL.

** 40% of 1930 leaves used from 25th to 35th days of experiment; from 35th day used 47.5% of 1930 leaves and 18.2% starch.

Ration No. 46 = 100 gm. No. 44 + 0.50 gm. cystine
Ration No. 48 = 100 gm. No. 47 + 0.05 gm. cystine
Ration No. 49 = 100 gm. No. 47 + 0.20 gm. cystine
Ration No. 54 = 100 gm. No. 47 + 0.50 gm. cystine
Ration No. 56 = 100 gm. No. 47 + 1.00 gm. cystine
Ration No. 60 = 100 gm. No. 57a + 0.50 gm. cystine
Ration No. 65 = 100 gm. No. 64 + 0.20 gm. cystine
Ration No. 68 = 100 gm. No. 67 + 2.00 gm. 1929 leaves
Ration No. 69 = 100 gm. No. 67 + 0.30 gm. cystine
Ration No. 71 = 100 gm. No. 70 + 1.00 gm. 1929 leaves
Ration No. 72 = 100 gm. No. 70 + 0.25 gm. cystine

It has been our experience that the minimum energy requirements for the maintenance of 60 to 70 gm. rats are met by an intake of approximately 30 gm. per week of the type of rations under discussion.

Our first evidence indicating that the crude protein of alfalfa is deficient in cystine was obtained from a group of 5 rats that had failed to maintain themselves over a period of 8 weeks on an average weekly intake of 50 gm. of ration 44. During the next 10 weeks the ration of two of these rats was supplemented with 0.5 per cent cystine (ration 46), resulting in an average weekly gain of 9.4 gm. During the next 5 weeks cystine was omitted with the result that these rats merely maintained their weights on an average weekly intake of 84 gm. per rat. The 3 rats which were continued on ration 44 made an average weekly gain of approximately 1 gm. on an average weekly intake of 62 gm. per rat. After having been restricted to ration 44 for about 3 months, the latter 3 rats were observed to be eating hair from each other.

Of 31 rats variously restricted to or fed *ad libitum* on rations containing added cystine (rations 48, 49, 54, 56 and 60) not a single rat failed to make better growth than that made by the best rat receiving similar rations without added cystine. The rat which made the poorest growth on a ration containing added cystine gained 25 gm. in 9 weeks on a ration (ration 48) containing only 0.05 per cent of added cystine. A group of 3 rats fed *ad libitum* on ration 49 with an average weekly food intake of 63 gm. per rat made an average weekly gain of 7.2 gm. per rat. Still another group fed ration 54 *ad libitum* over a period of 13 weeks made an average weekly gain of 9.8 gm. per rat. A group of 4 rats fed *ad libitum* on ration 56 over a period of 8 weeks with an average food intake of 76 gm. made an average weekly gain of 12.5 gm. per rat. In another experiment two carefully matched groups, each containing 3 rats, were restricted for 8 weeks to the same average weekly intakes (47 gm.) of rations 57a and 60. The average weekly gain per rat on ration 57a was 1.8 gm. while that on ration 60 was 5.6 gm.

Paired-Feeding Experiments

At about the time that our group experiments were completed, Mitchell and Beadles (7) pointed out the advantages of Armsby's paired feeding method for amino acid studies. This method aims to restrict individual animals to the *ad libitum* food intakes of matched individuals. It is particularly well adapted to obtaining results,which can easily be subjected to statistical analysis, from a limited number of animals. Considerable difficulty was at first encountered in our attempt strictly to equalize the food

TABLE II
DATA OBTAINED BY PAIRED-FEEDING METHOD

Pair No.	Ration No.	Ration	Initial Wt.	Final Wt.	Total gain.	Total food	Weeks	"Comparison of weekly gains."
1.	64	Control	65	95	30	373	7	4
	65	Cystine	67	96	29	336	7	3
2.	64	Control	60	71	11	332	7	2
	65	Cystine	60	87	27	345	7	5
3.	64	Control	60	97	37	349	7	1.5
	65	Cystine	60	115	55	364	7	5.5
4.	64	Control	63	91	28	330	7	0
	65	Cystine	60	110	50	337	7	7
5.	64	Control	56	93	37	358	7	3
	65	Cystine	56	107	51	340	7	4
6.	68	Control	82	97	15	412	8	1
	69	Cystine	80	105	25	376	8	7
7.	68	Control	84	98	14	356	8	2
	69	Cystine	84	113	29	364	8	6
8.	68	Control	85	93	8	355	8	2
	69	Cystine	85	112	27	366	8	6
9.	68	Control	95	108	13	376	8	3
	69	Cystine	86	112	26	366	8	5
10.	71	Control	105	121	16	461	6	1
	72	Cystine	100	145	45	460	6	5
11.	71	Control	107	125	18	448	6	1
	72	Cystine	107	155	48	448	6	5
12.	71	Control	72	91	19	321	6	1
	72	Cystine	69	97	28	319	6	5
13.	71	Control	64	78	14	291	6	1
	72	Cystine	60	93	33	293	6	5
14.	71	Control	65	78	13	316	6	1
	72	Cystine	65	99	34	316	6	5
15.	71	Control	72	88	16	328	6	1
	72	Cystine	68	104	36	328	6	5

intakes of pair mates while at the same time allowing *ad libitum* consumption by the rat consuming the smaller amount of food. In the case of 3 pairs the food intakes of pair mates differed by 5 per cent or more, the difference, however, being in favor of the control rats. Since the rejection of experimental results is a dangerous procedure, all pairs are included in Table II.

The results obtained in our paired feeding experiments are not strictly comparable with those obtained from our group experiments. In our earlier work with rations free from added cystine, it was usually found that rats would lose weight during the first few weeks on the experimental rations. This initial loss in weight was largely overcome in our paired feeding experiments by a one week preliminary feeding period on rations similar to rations 51 and 59.

The rations used are described in Table I. The results obtained are summarized in Table II. All pair mates were matched for litter and sex. Pairs 1 and 2 came from one litter; 3, 4 and 5 from one litter; 6, 7, 8 and 9 from one litter; 10 and 11 from one litter; and 12, 13, 14 and 15 from one litter. It should be noted that pairs chosen from the same litter appear to show a decided tendency to make similar gains.

Of 15 pairs of rats fed rations with and without added cystine, the rat receiving added cystine made the greater total gain in all pairs except one. In this case (pair 1) the growth curves were practically identical in spite of the fact that the control rat was allowed to consume approximately 10 per cent more food than was consumed by the cystine rat. It therefore appears highly probable that cystine has effectively supplemented alfalfa crude protein as supplied by alfalfa leaves.

A summary of the "comparison of weekly gains" (7) is given in Table II. Of 103 such comparisons 78.5 favored the cystine rat, a deviation of 27 from the ideal mid-value of 51.5. The standard deviation of 103 such comparisons ($\sqrt{.25\times103}$) is 5.08. The deviation of 27 is therefore approximately 5.3 times the standard deviation, an outcome which cannot reasonably be attributed to chance.

From the data in Table II one may readily find the differences between the gains of pair mates, and from these differences, the average weekly differences in gain. The arithmetical mean of these differences is 2.56 gm. in favor of the cystine rats and the standard deviation ($\sqrt{\sum d^2/N}$) is 1.30. The ratio of the mean difference of 2.56 to the standard deviation, "Student's" Z (8, 9) is 1.97, an outcome which again clearly indicates that the nutritive value of our control rations was definitely improved by small additions of cystine.

Discussion

It should be emphasized that the results presented in this paper have been obtained over a period of about 3 years with a variety of rations and with 4 distinctly different lots of leaves. There can be no reasonable doubt concerning the effectiveness of cystine in improving the nutritive value of rations (for rats) in which the protein is supplied by alfalfa leaves. Whether our results with rats can be confirmed with farm animals remains to be determined. Our results are in harmony with the well known tendency for the proteins of legume seeds to be deficient in cystine. Moreover, our paired-feeding results are of the same order as those obtained by Mitchell and Beadles (7) for foodstuffs which are apparently well suited to the digestive tract of the rat. Furthermore, we have scattered data obtained by a slight modification of the methods suggested by Bergeim (10) and by Heller *et al* (11), which indicate a coefficient of digestibility of the crude protein of alfalfa leaves of approximately 60 per cent. The fact that considerable growth was obtained in some of our group experiments where we fed rations containing added cystine, likewise indicates that poor digestibility was at least not the principal cause of the poor growth obtained on rations without added cystine.

There is perhaps no more vexing problem than that of food intakes and their interpretation. The writer is of the opinion that there is no evidence in our records which indicates that cystine affected the palatability of the rations fed. This opinion is in harmony with the findings of Beadles, Braman and Mitchell (12) as well as with the fact that there is no good reason to suppose that a small amount of added cystine would appreciably affect the palatability of a ration containing some 40 to 50 per cent of alfalfa leaves.

Summary

Numerous group and paired-feeding experiments have been conducted with rats, using rations containing alfalfa leaves to supply the proteins. These experiments are interpreted as demonstrating a deficiency of cystine in the mixed crude proteins of alfalfa.

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DETERMINATION OF BASAL METABOLISM OF THE ALBINO RAT FROM THE INSENSIBLE LOSS OF WEIGHT

By

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NUMEROUS methods have been described for the determination of the basal metabolism of the albino rat. A large majority of these have been discussed by Benedict and MacLeod (1) when they described a modification of the closed circuit method used in the Carnegie Nutrition Laboratory. All of the methods are complicated and require elaborate apparatus.

Since it has been shown by Benedict and Root (2) that the basal metabolism of man may be predicted from the basal insensible loss of weight, and Levine, Wilson, and Kelly (3) found that the maximal variation of the insensible loss of weight of infants from day to day under rigidly standard conditions rarely exceeded 10 per cent of the average, and Johnston and Newburgh (4) found the insensible loss of a man, over a period of 37 days, to vary within 10 per cent of the mean, it appears likely that the insensible loss of weight may be used to determine the basal metabolic rate of small animals.

The insensible loss of weight, as defined by Isenschmid (5), is equal to the weight of the water vaporized, plus the carbon dioxide eliminated, minus the oxygen absorbed. Johnston and Newburgh (4) showed that the water of vaporization constituted from 82 to 104 per cent of the insensible loss of weight in man when the respiratory quotients were 1.00 and 0.707 respectively. Therefore, if the respiratory quotient is known, the weight of the water vaporized and the heat lost by vaporization of water may be accurately found by the determination of the insensible loss of weight.

In a study of the metabolism of normal adults in a respiratory chamber, Benedict (6) found that the heat eliminated by vaporization of water varied between 19 and 30 per cent and averaged 22 per cent of the total heat production; Soderstrom and DuBois (7), also using a respiratory chamber, obtained averages from 23 to 27 per cent in normal boys, adults, and elderly men. Levine and Wilson (8) using the same method, reported

from 21 to 29 per cent, with an average of 24 per cent, for six normal children, and later (9) found, for four normal and six marantic infants, an average of 27 and 26 per cent. Johnston and Newburgh (4) studied the total heat elimination by man over a period of 37 days, and found that 27 to 28 per cent of the total heat was eliminated by vaporization of water.

The insensible loss of weight has been used to calculate the oxygen consumption in the method described by Haldane (10) for the determination of the total respiratory exchange in small animals.

Recognizing the need for a simple, yet accurate, method for the determination of the basal metabolic rate of the albino rat, the heat eliminated by water of vaporization, calculated from the basal insensible loss of weight, was compared with the basal heat production, determined by the gaseous exchange.

Method

The insensible loss of weight was obtained by means of a balance sensitive to 0.5 mg. with a load of 300 gm. The animal was placed in a screen wire cage, constructed by inverting a six-inch kitchen sieve over a six-inch tin pie plate, which was suspended to one arm of the balance. In the bottom of the plate was a clean white filter paper covered with a coarse mesh wire. The balance was placed upon a table, and the cage was suspended beneath the table and attached to the balance by a strong cord which passed through holes in the top of the table and the base of the balance cabinet (as illustrated by Levine, Wilson, and Kelly (3) for determining the insensible loss in infants). The space beneath the table was enclosed, however, with a glass door on one side. This prevented any interference from air currents; allowed a light to be directed upon the rat in order to induce sleep; and prevented any disturbance of the animal by movements of the operator when the balance was manipulated.

The animals were placed in the screen cage without food or water from 18 to 24 hours before the experiment. This permitted them to become accustomed to the smaller quarters; they therefore went to sleep more quickly at the time of the experiment than when placed in the screen cage just prior to the test. Immediately before the experiment the animal was removed, the cage was cleaned (a new filter paper was placed in the bottom of the cage) and weighed. The animal was returned to the cage, which was then suspended upon the balance. After the animal had been quiet for 15 to 20 minutes, the first weight was obtained and the time noted. The period for the determination of the insensible loss of weight varied between the time required to lose 50 to 1500 mg. of body weight. In some instances the

insensible loss was determined before the gaseous metabolism, and, at other times, afterwards. The majority of the time, however, it was determined at the same time as the respiratory exchange, by determining the weight and noting the time just before the cage was gently lowered into the respiratory chamber. After obtaining the respiratory gases, the cage was gently elevated and suspended from the balance. As soon as the animal was quiet, the weight and time were again noted. The animal was then re-

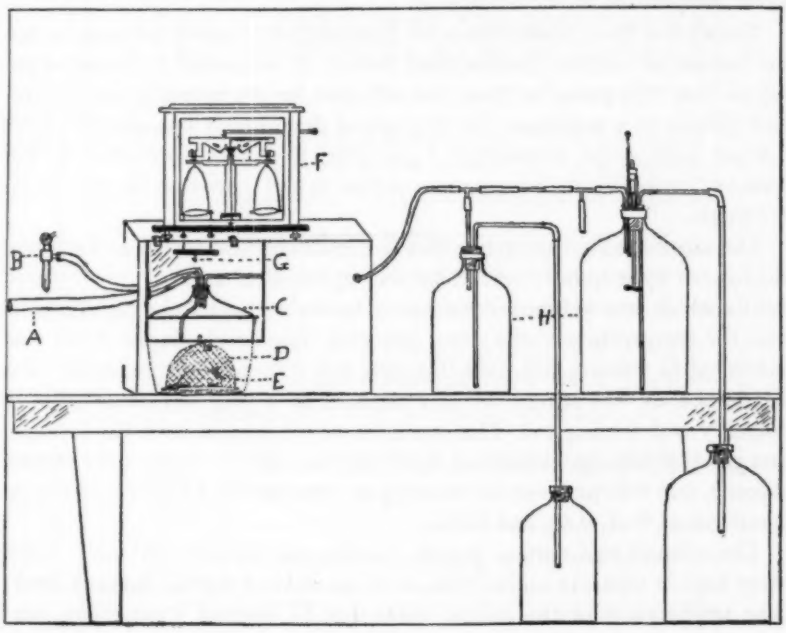

FIG. 1.

A. Hose for out of door air
B. Water suction pump
C. Vacuum desiccator
D. Screen wire cage
E. Animal
F. Balance
G. Hook for suspending cage to balance
H. Collecting bottles

moved and the cage weighed. It never changed in weight over 3 or 4 mg. unless the animal urinated or defecated, in which case the test was invalidated.

The gaseous metabolism was determined by a method similar to the one described by Prince (11), in which air was drawn through the animal chamber by the mechanism of a siphon and collected in a 20 liter bottle above the outflowing water, as shown in the diagram.

A large size vacuum desiccator was used for the respiratory chamber. The top was sealed with vaseline and could be easily removed, so that the cage containing the rat could be placed in the chamber or removed with only slight disturbance to the animal. Outside air, of a constant oxygen and carbon dioxide content, was first drawn through the animal chamber for thirty minutes at a rapid rate by means of a water suction pump. At the end of this time, the air in the chamber was repeatedly found to be of the same composition as the outside air.

The air was then drawn into a ten liter bottle for twenty minutes by the mechanism of a siphon (as described above). It was found by repeated analyses that this period of time was sufficient for the accumulation of carbon dioxide to a maximum for this rate of flow, which was usually about 1.0 per cent, never exceeding 1.5 per cent. It was then diverted to the twenty liter bottle at the same rate of flow as had been used for the smaller bottle.

The water used in the twenty liter bottle was triple distilled, and slightly acidulated by sulphuric acid. This was siphoned to another twenty liter bottle which was weighed immediately before and after the experiment,[1] and the temperature of the water recorded. The weight of the water was corrected to volume (12), and this gave the volume of air collected. The entrapped air was stirred for four minutes by a propeller attached to a mercury seal stirring rod. Then samples were analyzed with the Henderson gas analysis apparatus to an agreement of 0.04 per cent for the carbon dioxide, and 0.06 per cent for the oxygen. Alcohol checks gave respiratory quotients of 0.64, 0.65, and 0.653.

The animals had normal growth curves, and ate the diet[2] well. They were kept in separate cages which were cleaned and sterilized once a week. The temperature of the animal room was 27 degrees, Centigrade, constantly for three and one-half months.

Results

The influence of the respiratory quotient upon the percentage of the insensible loss of weight that is composed of water of vaporization is well illustrated by Rats No. 7 and No. 9. The data are shown in Table I.

Rat No. 9, on April 22, 1930, had a respiratory quotient of 0.707, and the water vaporized equalled 102.1 per cent of the insensible loss of weight;

[1] The weights were determined on a pair of silk scales (3) to an accuracy of 5 gm. for this weight.

[2] Diet consisted of corn starch 48 per cent, lard 25 per cent, dried pulverized beef 20 per cent, cod liver oil 3 per cent, and salt mixture 4 per cent. For vitamin B, four Harris yeast tablets were given daily. The salt mixture was that described by Osborne and Mendel.

TABLE I

HEAT PRODUCTION CALCULATED FROM INSENSIBLE LOSSES

Rat No.	Date	Wgt.	Age	Sex	Temp. °C	O_2 gm.	CO_2 gm.	R.Q.	Ins. Loss 24 hrs. (gm.)	Heat produc. 24 hrs. (Cal.)	Heat loss by vaporization of water	Per cent Heat loss by vaporization of water
5	4–18–30	184	4½	F	31	4.793	4.718	0.716	6.450	15.76	3.85	24.43
5	5–18–30	225	5½	F	28	5.209	5.125	0.715	7.810	17.12	4.59	26.80
6	4– 5–30	168	4	F	30	4.516	4.589	0.739	6.570	14.94	3.78	25.30
6	4–29–30	201	5	F	31	6.419	6.477	0.734	7.400	21.20	4.27	20.63
7	2–25–30	241	5	M	27	7.019	7.581	0.780	9.600	23.47	5.26	22.42
7	3–22–30	205	6	M	28	5.943	5.893	0.721	8.490	19.56	4.96	25.35
7	3–29–30	210	6	M	28	6.304	6.230	0.719	8.280	20.74	4.86	23.41
7	4–24–30	244	7	M	25	5.971	5.774	0.703	8.540	19.56	5.08	25.99
7	5–18–30	269	8	M	27	6.319	6.534	0.752	8.932	20.97	5.07	24.19
9	2–26–30	232	5	M	29	6.247	6.135	0.714	9.294	24.64	5.47	22.21
9	3–25–30	225	6	M	30	5.752	5.626	0.711	8.020	18.89	4.74	25.10
9	3–30–30	234	6	M	26	7.195	7.540	0.744	9.270	24.37	5.19	21.32
9	4–22–30	267	7	M	27	6.734	6.541	0.707	9.230	22.09	5.48	24.83
10	4– 1–30	205	6	M	28	5.477	5.421	0.719	6.720	18.03	3.94	21.88
10	5–14–30	285	8	M	29	7.040	6.784	0.801	8.640	23.05	5.18	22.45
10	5–21–30	297	8	M	30	6.875	6.670	0.705	8.926	22.55	5.31	23.56
11	4–25–30	475	8	M	30	12.456	12.043	0.702	16.000	40.85	9.55	23.93
12	5– 4–30	150	4	M	29	5.499	5.369	0.710	8.170	18.05	4.83	26.76
13	5– 2–30	161	4	F	29	6.065	6.200	0.743	7.319	20.90	4.18	20.81
13	5– 4–30	164	4	F	31	5.674	5.509	0.706	8.800	18.61	4.64	24.91

while, with Rat No. 7, on February 25, 1930, with a respiratory quotient of 0.78, the water vaporized equalled 94.1 per cent of the insensible loss. With each gram of water vaporized at 28 degrees, Centigrade, there are 0.582 calories given off.

Benedict and MacLeod (1) state that "with rats, the respiratory quotient is invariably not far from 0.72 or 0.73 when food has been withheld for sixteen hours." Mitchell and Carman (13) report an average of 0.739, while, in our series, the average respiratory quotient was 0.719. With the respiratory quotient averaging 0.719, there would be 100.8 per cent of the insensible loss as water of vaporization, as illustrated by Rat No. 7 on March 29, 1930. If the insensible loss were to be considered 100 per cent water, the calories lost by vaporization of water would be 4.82, whereas there were actually 4.86 calories, a difference of 0.04 calories. When the albino rat has fasted for from 18 to 24 hours, one may assume that the respiratory quotient is approximately 0.72 and that the insensible loss is composed of 100 per cent water of vaporization with the addition of an error of only 0.83 per cent.

The optimum temperature for the minimum heat production of the albino rat has been shown by Benedict and MacLeod (1) to be 28° C. Our environmental temperatures ranged between 25 and 31° C. without there being any constant change in the heat production or the percentage of heat lost by vaporization of water.

The total heat production of our animals compares favorably with that reported by Mitchell and Carman (13), and is somewhat lower than that reported by Benedict and MacLeod (1). The fact that our animals were asleep, and that they were unable to observe any movements of the operator during the experiment may account for our lower metabolic rates. Another thing that is thought to have lowered the heat productions is the fact that the animals had been in a constant environmental temperature of 27° C., for from two to three and one-half months. Animals No. 12 and No. 13, however, were brought in from the animal stock house, where the environmental temperature had ranged between 18 and 20° C. and their heat production compared favorably with that of the other animals.

The amount of heat lost by vaporization of water from the skin and lungs of the albino rat was remarkably constant as compared to the weight of the animal and the total heat produced. The percentage of heat lost by vaporization of water averaged 23.81 and varied between 20.63 and 26.80 per cent, with a maximal variation from the average of 13.3 per cent.

With Rat No. 5 on April 18, 1930; No. 6 on April 5, 1930; No. 10 on April 1, 1930 and No. 7 on March 22 and March 29, 1930, the total heat pro-

duction decreased markedly, due to the frequent fasting periods that accompanied the metabolic tests. They lost body weight and became quite thin, in accord with the experience of Benedict and MacLeod (1). It is interesting to note that the percentage of heat lost by vaporization of water by those thin animals was very close to the average.

An obese rat, No. 11, which Doctor C. A. Lilly very kindly allowed us to use, (one month older than our animals, but weighing 505 gm. before the twenty-four hour fasting period) showed a percentage of heat lost by vaporization of water very near the average. The lowered heat production in the female has been emphasized by Mitchell and Carman and by Benedict and MacLeod. This was confirmed in our series of experiments. The percentage of the total heat production that was lost by vaporization of water, however, did not show any constant variation between the two sexes.

Summary

1. The heat lost by vaporization of water and the heat production of the albino rat, in the basal state, have been determined under standard conditions of 18 to 24 hours without food or water and an environmental temperature of from 25 to 31° C.

2. The heat lost by vaporization of water averaged 23.81 per cent of the basal heat production of the albino rat, with a maximal variation of 13.3 per cent from the average, which held for normal, thin, and obese albino rats of both sexes.

3. Under these standard conditions, the respiratory quotient of the albino rat is about 0.72, and the water of vaporization is approximately 100 per cent of the insensible loss of weight. Insensible loss of weight per hour $\times 0.58 \times 100/25 \times 24$ equals calories per 24 hours.

We wish to express our gratitude to Doctor L. H. Newburgh for his interest in this work, and for his many helpful criticisms and suggestions.

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FACTORS WHICH DETERMINE RENAL WEIGHT*

**XII. THE NITROGEN INTAKE AS VARIED BY THE ADDITION OF UREA TO THE DIET

By

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IN THE preceding papers of this series (MacKay, MacKay and Addis, 1928; MacKay and MacKay, 1930) it was shown that when casein was the food protein the weight of the kidneys could be predicted from measurements of the casein consumption (casein intake = 0.0183 renal weight − 2.75). But the nitrogen of casein leaves the body almost entirely as urea and if this is the only mechanism underlying the casein-kidney weight relationship the same curve should be reproduced when urea is substituted for equivalent amounts of casein in the food. The experiments detailed in this paper were designed to test this hypothesis.

The first experiments of which we are aware, in which an attempt was made to increase the size of the kidneys by feeding urea in large amounts, were carried out in this laboratory by Addis and Shevky (described by Emge, 1921) on rats with[1] negative results in so far as any renal enlargement was concerned. Hinman (1923) also found no change in the size of the kidneys of rats following prolonged urea administration. At the time of their preliminary report dealing with the renal hypertrophy which ensues on high protein diets Osborne, Mendel, Park and Winternitz (1925) believed that comparable changes in renal weight could be produced by the inclusion of large quantities of urea in the diets. In their later experiments (1927) urea fed in large amounts, equivalent to the protein nitrogen concentration of the other diet, did not seem to lead to an equivalent enlargement of the kidneys.

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[1] This is somewhat surprising in view of the results reported here. The explanation seems to be in the fact that the rats were kept on the urea diet for a year and at the age they were killed the caloric requirement would have decreased and hence the urea intake would have been much smaller than at an early age (MacKay and MacKay, 1930). With the small number of rats which were used a minor increase in renal weight might have been easily overlooked.

TABLE I

Diet No.	No. Rats in group	Body length	Body weight (corrected)	Body surface	Heart Weight		Liver Weight		Kidney Weight	
					Actual	mgm. per 100 sq. cm. body surface	Actual	gm. per 100 sq. cm. body surface	Actual	mgm. per 100 sq. cm. body surface
		mm.	gm.	sq. cm.	mgm.	mgm.	gm.	gm.	mgm.	mgm.
	70 Days Old									
2	25	192	170	348	640	184	8.42	2.42	644	185
8	22	192	176	355	644	181	8.81	2.48	698	196
9	22	216	181	363	644	177	7.88	2.17	744	206
10	22	196	189	374	703	188	8.60	2.30	879	235
11	24	—	162	341	613	180	8.32	2.44	839	249
	400 Days Old									
2	22	231	367	581	949	163	12.89	2.21	1035	178
8	23	—	375	590	957	162	11.52	1.95	1031	175
10	10	—	333	543	953	175	11.63	2.14	1152	213
11	24	—	294	501	906	181	10.35	2.07	1030	209

TABLE II

Diet No.	Per Cent protein	Per Cent urea	Intake per 100 sq. cm. body surface per day				Mgm. kidney per 100 sq. cm. body surface				
			*Food	Protein	Urea	Urea nitrogen calculated as protein	Observed	**Calculated from protein intake	Deviation from observed	**Calculated from nitrogen intake as protein	Deviation from observed
			gm.	gm.	gm.	gm.			per cent		per cent
	70 Days Old										
2	18.0	0.0	3.39	0.61	0.00	0.00	185	183	− 1.1	183	− 1.1
8	18.0	4.0	2.90	0.52	0.12	0.34	196	179	− 8.7	197	+ 0.5
9	18.0	11.0	3.20	0.68	0.35	0.99	206	187	− 9.3	242	+17.5
10	31.3	11.0	3.20	1.00	0.35	0.99	235	205	−12.8	259	+23.0
11	18.0	18.0	4.74	0.85	0.85	2.42	249	197	−20.8	329	+32.1
	400 Days Old										
2	18.0	0.0	2.11	0.38	0.00	0.00	178	171	− 3.9	171	− 3.9
8	18.0	4.0	1.50	0.27	0.06	0.17	175	165	− 5.7	176	+ 0.6
10	31.3	11.0	2.11	0.66	0.23	0.65	213	186	−12.7	222	+ 4.2
11	18.0	18.0	1.82	0.33	0.33	0.95	209	168	−19.6	220	+31.0

* Determined from the average of the last 10 days of the experiment.

** Calculated by formula: *Protein Intake=0.0183 Renal Weight−2.75* (MacKay, MacKay and Addis, 1928).

The experimental methods have been described (MacKay and MacKay, 1927). The special diets which were used were prepared by replacing a portion of the cornstarch in diets which have been used before (MacKay, MacKay and Addis, 1928) with urea. This raised the nitrogen content of the diets accordingly and reduced their caloric value from 3 to 7 per cent. Growth during the period of observation was poorer on the urea containing diets in proportion to their urea content than on the control diet which

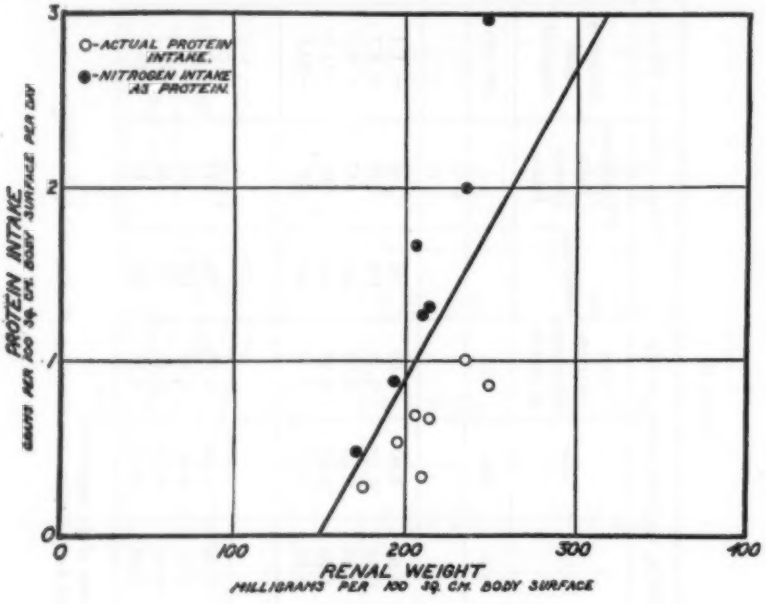

FIG. 1.

was free of this substance. Two groups of experiments were carried out, both on male rats of exact and known age. In one of these the rats were fed the special diets from 44 to 70 days of age and in the other from 346 to 400 days of age. These experiments were identical, except for the diet given, with those described for the protein diets. At 70 and 400 days of age respectively the rats were killed and anatomical measurements made. The average body weight and organ weights for each experimental group are presented in Table I.

It is obvious that the addition of urea to the diet is followed by a significant increase in renal weight. Our special interest is whether or not this is of the same order of magnitude as the increase in the size of the kidneys

which would be produced by the ingestion of similar quantities of nitrogen in the form of casein. To answer this question the data have been analyzed in Table II and Figure 1. In no experiment is the increase in renal weight as great in relation to the urea nitrogen ingested as the formula (MacKay, MacKay and Addis, 1928; MacKay and MacKay, 1930) demands for an equivalent amount of protein nitrogen. The difference is more significant than some of the actual figures indicate for we have assumed 100 per cent absorption from the intestinal tract of the nitrogen contained in both the urea and the protein. It seems certain that the urea nitrogen would be more completely utilized in so far as absorption is concerned than the protein nitrogen. We must conclude then that nitrogen in the form of urea added to the diet has less effect in increasing the weight of the kidneys than nitrogen in the form of casein.

Summary

The addition of urea to the diet leads to an increase in the weight of the kidneys of male albino rats 70 and 400 days of age. This increase in renal weight is less than that produced by the same nitrogen consumption obtained by the administration of protein instead of urea.

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GLYCOGEN FORMATION AND RESPIRATORY QUOTIENTS IN RATS FED EXCLUSIVELY ON FAT*

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IT IS generally agreed that the mammal is able to synthesize carbohydrate from protein, either in the normal or diabetic organism, and from glycerol in the diabetic state. Unanimity of opinion is lacking that either portion of the fat molecule may be converted into glucose in the normal organism. Pflüger (1907) was unable to recover any liver glycogen after feeding a dog 40 days on a pure fat diet. Bodey, Lewis and Huber (1927) were unable to demonstrate a deposition of glycogen in the liver of the rat fed 48 hours on butter fat. But Takao (1926), Burn and Ling (1928) and Magnüsson (1929) have pointed out that the rat on such a diet may deposit as high as 3 per cent liver glycogen. This deposition occurred only after 2 to 3 days of such feeding and increased thereafter. In all of these researches no analyses were made of the body glycogen content. These values may have decreased sufficiently to account for the increased liver deposits. Protein may also have been the source. But granted that the source is the fat administered, there is no way of telling, except by analogy with the diabetic, from which portion of the molecule the glycogen was derived.

If the fatty acid portion of the fat molecule were converted into carbohydrate at a sufficiently rapid rate, then at some time during its metabolism the R. Q. should exhibit a pronounced lowering, especially if the preformed glycogen had been reduced to a minimum by a preliminary starvation period and the carbohydrate were stored as glycogen. If glycerol were the cause of such sugar, the effect on the R. Q. would be negligible.

The object of the following experiments was to investigate the effect of fat feeding upon 1.–liver glycogen, 2.–total body glycogen, and 3.–the R. Q. during the absorption and metabolism of the ingested fat. From the data derived from these determinations it should be possible to make an accurate comparison between the administered fatty acids and glycerol and the glycogen deposition if such there should be.

* Taken from a thesis submitted to the University of Rochester in partial fulfillment of the requirements for the Doctorate of Philosophy.

General Procedure

Thirty rats, divided into three control and three experimental groups, were used. Experimental and control groups were started together, so as to avoid differences due to season of the year. Young albino rats of 150 to 200 grams in weight and as far as possible of the same age and sex were placed on a constant diet for preliminary periods varying from a few days to six weeks. They were in the experimental room at least 24 hours previous to the start of the metabolism experiment. The rats were then starved approximately 24 hours. The control animals after starving one day were killed by a blow on the head and examined for their free sugar and glycogen content. The experimental group, after 24 hours fasting, was given butter fat. This was prepared by melting the butter, decanting it from its water three times and then filtering twice through a hot funnel to remove traces of casein. To the butter fat was added 3 per cent of Osborne and Mendel (1919) salt mixture. The rats were in special metabolism cages in which there was no scattering of food and no mixing or washing of the feces with urine. Daily records were kept of the weights of the animals, butter eaten and the urinary nitrogen excretion. The rats either ate the fat (group I) or were given it by stomach tube (groups II and III). They were kept in the metabolism trains 70 to 80 per cent of the time, being removed, as a rule, only for the passing of the stomach tube and urine collection. Two to three feedings per day were given and R. Q.'s were obtained immediately following and at varying periods after fat ingestion. One rat was starved 8 days and its carbohydrate content noted. Two others were given the saponifiable fraction contained in the butter fat. One rat was fed the potassium soaps obtained from butter fat. Experimental periods lasted from 3 to 9 days. The rats were then killed and analyzed in the same manner as the controls. Control sub-groups are numbered Ia, IIa and IIIa, experimental subgroups Ib, IIb, and IIIb.

Analytical Procedure

Urinary Nitrogen. The urine was collected from the cages in 24-hour periods and analyzed by the macro-Kjeldahl method.

Carbohydrate Content. It was felt that a glycogen analysis on the whole animal was not sufficient to recover all the sugar in the carcass. Cori and Cori (1925), Palmer (1917) and others, have reported considerable amounts of free sugar in preparations analyzed with the utmost care to prevent glycogenolysis. Hence, different analytical procedures were tried in the three groups of rats in the effort to obtain the total sugars in the carcass, the same method in all cases being applied to controls and experimental sub-groups. In all three groups the initial procedure consisted in stunning the rat, immediately opening the abdomen and quickly removing the liver. The rat was then skinned and the carcass passed three times through a fine meat grinder. In group I the liver glycogen was determined by the method of Bierry and Gruzewka (1912), the hydrolysis being carried out in a boiling water bath and the proteins precipitated by the method of Folin-Wu (1928).

Applying this method to two liver brei mixtures, it was found to recover an average of 102 per cent of added C. P. glycogen. In groups II and III the liver glycogen was determined by Büttner's (1926) modification of Pflüger's method. In group III the glycogenolysis had previously been stopped by boiling the tissue in water and the free sugar extracted in 70 per cent alcohol. In group I the hide was analyzed by the method of Bierry and Gruzewka and the carcass free sugar by the method of Palmer (1917). In groups II and III the carcass and hide were analyzed together. Both were plunged into boiling water. After 15 minutes on a hot plate the beakers were placed on a steam bath and brought to a small volume. Alcohol, 95 per cent, was added to give a resultant concentration of 70 per cent by volume. The samples were placed in the ice box over night and next morning passed through a fine filter with repeated washings into volumetric flasks. Aliquots of the filtrates were evaporated to dryness on a steam bath, the residue taken up in 2.2 per cent HCl and hydrolyzed three hours in a boiling water bath. After neutralization the proteins were precipitated and the sugar determined. The residue on the filter paper and in the beaker were combined and analyzed according to the method of Pflüger (1910). The final sugars in all cases were rendered protein free by the method of Folin-Wu (1928) and determined by the Folin method (1929). In group I the true sugars were determined using Somogyi's technique (1928).

Respiratory Quotients. These were obtained on groups II and III by use of metabolism trains very similar to those described by Haldane (1892). Vacuum desiccators were used as the animal chambers. The animal was enclosed in a small wire cage contained in these and suspended over a tray for the collection of urine. Each set of absorbers was checked by another set immediately following it. These rarely showed any change in weight. In group IIb these experiments were carried out at a temperature of about 20°C; in group IIIb the experiments were done in a constant temperature room at 27 to 28°C. It is obvious that the activity of the rats in these chambers over long periods could not be controlled. This, however, has no influence on the R.Q.'s obtained. It only affects the total heat production. In all cases the activity was reduced to a minimum by allowing an electric light bulb to shine on the desiccators.

TABLE I

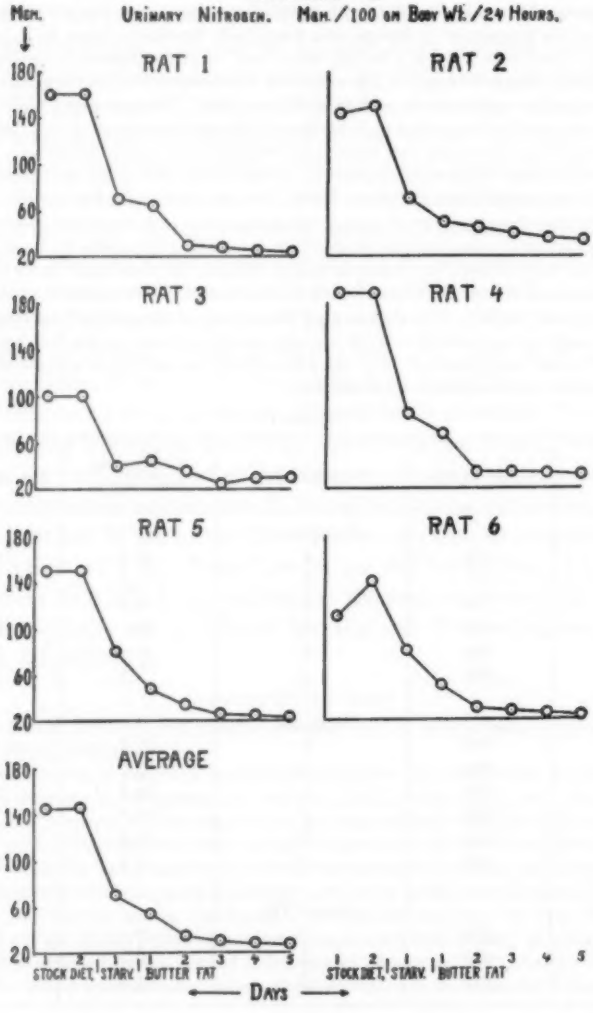
No. Rat	Initial weight	Days fat diet	% Decrease body weight	Fat intake gms.
		Group Ib		
1	170	4	2.9	19.6
2	171	4	2.9	17.9
3	164	4	4.2	13.5
4	180	4	0	26.6
5	200	4	0	29.6
6	200	4	5.5	18.9
		Group IIb		
1a*	198	8	25.7	0
4	204	6	11.7	21.6
5	173	7	10.4	26.1
6	174	8	5.7	28.7
7	222	3	5.8	16.8
8	188	8	8.5	32.6
		Group IIIb		
1	242	3	0	21.7
2	212	2	0	11.8
3	235	3	1.3	19.5

* Starved

Experimental Results

Body Weight. The animals lost weight in groups Ib and IIb. In group IIIb, in which the caloric intake exceeded the calculated caloric output, there was very little change in weight. Table I shows these weight changes together with the fat ingestion for all the rats which received fat.

CHART 1



Nitrogen Excretion. The urinary nitrogen was determined for all the experimental animals. Chart 1 shows the behavior of the total N excretion in the fat-fed rats of group Ib, this being typical of the other groups. Not all the curves obtained were as smooth but all showed the same marked diminution in excretion from day to day. The nitrogen excretion amongst members of the three groups, (Table II, column 10), is fairly constant. In group IIb, rat 1a was starved 8 days and its urinary nitrogen determined as well as its carbohydrate content. The nitrogen value, 45.8 mg. per 100 grams per 24 hours, is considerably higher than that for any of the fat-fed rats except rats 1 and 2, group IIIb. The sparing of body protein possibly illustrated here is due either to ingested fatty acids or glycerol, or both.

Tissue Analyses. These values for all three groups are presented in Table II. The average values per 100 grams of rat for the control sub-groups are 167, 147 and 126 mgm., with an average value for all three of 148 mgm $\pm$ 20 mg. Cori and Cori (1928) give an average of 143 mg. $\pm$ 11 mg. for 24-hour starved rats analyzed by Pflüger's method.

In sub-group Ib only 4 of the 6 fat-fed rats showed an appreciable rise in liver glycogen. It just so happens that these rats ate the largest amount of butter fat (Table I). When the total carbohydrate is expressed as mg. per 100 grams of rat, the average for the fat-fed rats on the basis of true sugar exceeds the controls by about 10 per cent. Expressed as total reducing substance, there is a loss of 4.8 per cent. If only the 4 rats which showed deposits of liver glycogen are averaged, there is an increase of 1 per cent or 22.5 per cent on the basis of the total reducing substance or true sugar respectively.

The rats in sub-group IIb are comparable with sub-group Ib. Rats 6, 7, and 8 show deposits of liver glycogen; rats 4 and 5 do not. All except rat 6 had diarrhea on the first day of feeding. Rats 4 and 5 appeared quite sick and died at an unknown time. This interval of time from death to analysis no doubt accounts for their low glycogen content. Taken together this group showed an increase of 25 per cent in total sugar content. If only those rats are averaged which deposited glycogen in their livers, the percentage increase is 41. These findings indicate in a more definite manner than in group Ib the absolute increase in body glycogen in such preparations. All three rats in sub-group IIIb exceeded the control sugar values, the increase being 39 per cent. Rat 1a, Group IIb, which was starved 8 days showed a glycogen value for the liver quite similar to the controls.

To obtain a rough idea as to the speed with which this glycogen was stored, one rat was starved 40 hours and given 2 grams of butter fat by stomach tube. Eight and one-half hours later, the liver glycogen value was

TABLE II

GLYCOGEN FORMATION AFTER FAT FEEDING

(ALL GLYCOGEN ANALYSES CALCULATED AS FREE SUGAR AND EXPRESSED AS MGM. PER 100 GM. RAT)

No.	Wt. rat gms.	Liver % body weight	Liver glycogen and free sugar	Carcass glycogen and free sugar	Total Reducing Sub.	No.	Wt. rat gms.	Liver % body weight	Urine N	Liver glycogen and free sugar	Carcass glycogen and free sugar	Total Reducing Sub.
Group Ia, Controls					Dec. 1929	Group Ib, Butter Fat						
1	200	2.6	10.7	138	149	1	165	2.7	35.8	43.9	157	201
2	188	2.9	11.3	180	191	2	166	2.7	41.3	11.1	120	131
3	195	3.2	13.9	172	186	3	157	4.1	33.7	15.2	134	149
4	176	2.4	15.7	161	177	4	183	2.7	37.2	42.2	123	165
5	204	2.8	12.1	122	134	5	201	3.7	33.4	59.0	107	166
						6	189	2.8	29.3	37.5	107	145
Av.	193	2.8	12.7	155	167±21		177	3.1	34.6±3	34.8	126	159±18
Av. (True sugar by yeast fermentation)			9.2	105	114±16					31.8	97	129±18
Group IIa Controls					Feb. 1930	Group IIb, Butter Fat						
1	169	3.5	4.6	150	155	1a*	147	3.0	45.8	10.9	150	161
2	154	2.7	2.3	124	126	4	180	5.2	39.3	4.8	126	131
3	218	3.0	10.8	106	117	5	155	5.3	44.2	12.0	152	164
4	158	3.0	4.7	147	152	6	164	3.6	34.8	67.2	169	236
5	146	3.1	6.4	171	177	7	210	4.3	41.3	32.8	156	189
6	186	3.2	11.3	143	154	8	173	3.9	43.5	48.5	149	198
Av.	171	3.1	6.7	140	147±17		172	4.2	40.6±2.8	33.1	151	184±29
Group IIIa, Controls					May 1930	Group IIIb, Butter Fat						
4	245	2.7	6.6	112	119	1	246	3.7	48.6	84.0	103	186
5	198	2.5	9.1	118	127	2	211	4.4		46.6	134	181
6	212	2.7	15.5	117	133	3	232	3.2	37.0	43.0	114	157
7	255	3.1	12.1	113	125							
Av.	228	2.8	10.8	115	126±4		230	3.7	43.7±5	57.9	117	175±12
							* starved					

3.12 mg. per gram of liver, a figure agreeing with the controls. Burn and Ling were also unable to obtain any appreciable glycogen deposition until the third or fourth day on such a diet.

No success was attained by feeding the saponifiable fraction of butter fat to two rats. The feeding was attended with violent diarrhea and was discontinued after 2 days. No analyses were made. The fraction fed was extracted as follows; butter fat was saponified with an excess of 25 per cent NaOH. This was acidified with HCl and the mixture extracted with petroleum ether. The solvent was later removed.

One rat was starved 96 hours and then allowed to eat the potassium soaps formed from the above preparation, the intake averaging about 4 grams a day. The liver glycogen was found to be 2.32 mg. per gram. From this single experiment it would seem that the glycogen deposition observed in the other two groups was due to the ingestion of the glycerol fraction rather than to the fatty acids. Further experiments are being carried out to clarify this point.

The R. Q.'s are set forth in Chart 2. All animals except rat 8 were kept in the metabolism chamber the major portion of the time. Of all the quotients obtained, only one—a 0.634 on rat 5—may be regarded as indicating sugar formation with fatty acids as its source. Ten figures lie between 0.66 and 0.70. These occur during the early hours of fat absorption and are due without doubt to a transient acidosis and ketonuria.

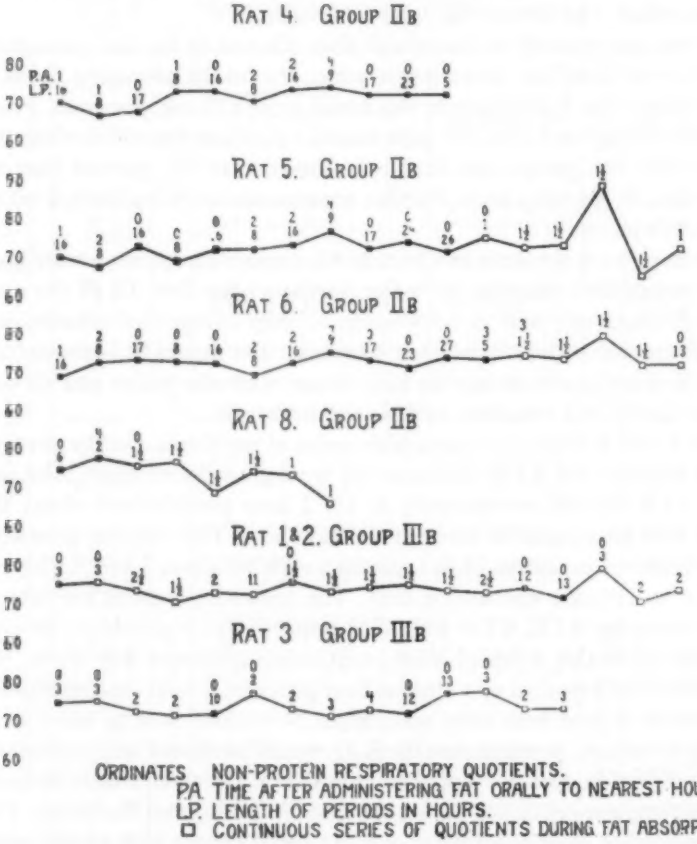
Rats 5 and 6 display a comparable series of quotients. On the seventh day of fat ingestion 2.5 cc. of butter fat were given by stomach tube and the R. Q.'s followed continuously in 1-1/2 hour periods from about the second hour after ingestion through the tenth hour. This was also done with rat 8. With the exception of one quotient each with rats 5 and 6, there is nothing to indicate conversion here. The remaining figures for rats 4, 5 and 6 average 0.726, 0.719 and 0.712 respectively. It should be pointed out that when this series of short, continuous quotients was made, the rats had already worked up a store of liver glycogen. Under such conditions any glucose formed from fatty acids might be oxidized in large measure as quickly as formed, in which case the R. Q. would be that of fat combustion.

This objection does not apply to group IIIb. Here continuous series of observations were obtained during the first fat feeding and thereafter. The basal quotients (post-absorptive) of 0.743 and 0.748 are high as compared with others obtained at a room temperature of 20°C., these averaging about 0.71 (not reported in this paper). The subsequent quotients during fat absorption average 0.729 and 0.732, respectively, figures both higher than in group IIb. There is no quotient below 0.700. In this series, if fatty acids

were being converted into sugar, then during the peak of absorption one might expect low R.Q.'s, indicating conversion and storage. Subsequently the quotients might increase to much above a fat level, indicating oxidation of the sugar formed. However, these quotients in general lend no support to the idea that the rat can store glycogen derived from ingested fatty acids.

CHART 2

Respiratory Quotients After Fat Feeding.



ORDINATES NON-PROTEIN RESPIRATORY QUOTIENTS.
PA. TIME AFTER ADMINISTERING FAT ORALLY TO NEAREST HOUR.
LP. LENGTH OF PERIODS IN HOURS.
□ CONTINUOUS SERIES OF QUOTIENTS DURING FAT ABSORPTION.

In Table III appears the heat production of the rats in groups IIb and IIIb, with its apportionment to carbohydrate, fat and protein. The total heat output per 100 grams of rat, per 24 hours, in group IIb of rats 4, 5 and 6, is fairly constant, the average being 19.12 ± 4.9 per cent. In group IIIb,

TABLE III
SUMMARY OF HEAT PRODUCTION

No. rat	Time of experiment	% time in metabolism chamber	Av. non-prot. R.Q.	Calories/100 gm. body wt.				Caloric intake/100 gms.	Total Cal. 100 gm./24 hours
				Carboh.	Prot.	Fat	Total		
		Group IIb—on Basis of Time spent in Metabolism Chamber							
4	140.85	79.0	0.707	3.29	6.13	111.19	120.60	119.0	20.54
5	174.0	78.0	0.718	4.06	6.99	125.07	137.23	164.6	18.92
6	186.0	73.0	0.720	6.51	7.12	125.05	138.83	179.8	17.91
		Group IIb—on Basis of Total Time of Experiment							
4	140.85	79.0	0.707	4.17	6.13	134.5	153.0	119.0	20.54
5	174.0	78.0	0.718	5.21	6.99	160.5	176.0	164.6	18.92
6	186.0	73.0	0.720	8.91	7.12	171.2	190.0	179.8	17.91
		Group IIIb—on Basis of Time Spent in Metabolism Chamber							
1+2	83.0	72.0	0.729	1.99	3.88	28.55	34.45	73.9	13.35
3	83.5	70.5	0.732	2.57	2.66	25.93	31.19	75.8	12.62
		Group IIIb—on Basis of Total Time of Experiment							
1+2	83.0	72.0	0.729	2.76	3.88	39.70	47.90	73.9	13.35
3	83.5	70.5	0.732	3.65	2.66	36.80	44.20	75.8	12.62

the average is lower, 13.00 ± 2.7 per cent. This is due, no doubt, to the higher environmental temperature. Only in group IIIb, did the calculated caloric intake exceed the calculated caloric output.

In Table IV an attempt is made to balance the deposit of glycogen and its oxidation against its various possible derivatives. Any R. Q. exceeding 0.707 was regarded as indicating sugar oxidation. Such an arbitrary mean is not wholly valid, for the quotient of the complete oxidation of butter fat is more nearly 0.720. On this basis there would be little or no sugar oxidized in these experiments as determined by the non-protein R. Q. What fraction of the total metabolism is due to the oxidation of ingested fat, as contrasted with body fat, cannot be told in these experiments. If the calculation be made in groups IIb and IIIb, however, the sum of the sugar oxidized as determined by the R. Q. plus the increase in body carbohydrate per 100 grams, averages 45 and 60.9 per cent respectively of that derivable 1.–from the ingested glycerol, 2.–plus that from protein metabolism and 3.–plus that from preformed glycogen. The average for both groups is 53.0 per cent. One and two-tenths, and 4.2 per cent, or an average of 2.7 per cent of the sugar derivable from the ingested glycerol, plus that due to protein, is recoverable as body glycogen in the groups IIb and IIIb. If, however, it is assumed that the rats in these two groups oxidized the same percentage of the three foodstuffs while out of the metabolism chamber as in it, and this period is taken into the calculation, then in groups IIb and IIIb, the values for oxidation plus deposit average 60 and 83 per cent respectively.

The marked depression of the urinary nitrogen excretion on the fat diet as compared with the previous 24-hour starvation period, is well brought out in these experiments (Chart 1). In the three groups the depressing action is 66 and 44 per cent in the first two groups, while it rose slightly in the third. It is very probable that a small fraction of sugar from protein has contributed to the observed formation of body glycogen. Such a process would tend to lower the R. Q. but in reality in these experiments the process is too small to have any effect. The ratio of actual sugars deposited to potential protein sugar is 11.5, 8.2, 0, 1.7, and 8.8 per cent respectively in rats 1 and 2 (which were in the same cage), 3, 4, 5, 6.

Discussion

From the preceding experiments a small but seemingly measureable deposit of glycogen in rats on a pure fat diet is the chief finding. The following table summarizes the carbohydrate analyses of all groups.

This deposit of glycogen was not attended with a depression of the R. Q.,

TABLE IV

GRAMS CARBOHYDRATE PER 100 GRAMS BODY WEIGHT

1	2	3	4	5	6	7	8	9	10
No. Rat	Carboh. oxidized from non-prot. R.Q.	Final body carboh.	Total carboh. (2+3)	From glycerol fed, gms.	Carboh. from prot.	Initial body carboh.	Total carboh. (5+6+7)	2+(3−7) 100 / 5+6 %	(3−7) 100 / 5+6 %
Group IIb									
4	0.802(1.015)	0.131	0.933(1.146)	1.126	0.860	0.147	2.133	39.0(51.0)	0
5	0.890(1.140)	0.164	1.054(1.271)	1.557	0.980	0.147	2.684	35.7(45.5)	0.63
6	1.589(2.169)	0.236	1.825(2.300)	1.780	1.007	0.147	2.934	60.2(81.0)	3.00
Group IIIb									
1+2	0.486(0.670)	0.183	0.670(0.853)	0.762	0.550	0.126	1.438	41.5(55.4)	4.6
3	0.627(0.890)	0.157	0.784(1.047)	0.444	0.376	0.126	0.946	80.0(111.0)	3.8

The figures in parentheses are based on the total time of the experiment.
The other figures are based on the percentage of time the rat was in the metabolism chamber.

such as might indicate conversion of fatty acids to sugar. This effect of fat feeding on body carbohydrate may be attributed to at least four causes; 1.–tissue dehydration, 2.–sugar formation from protein, 3.–sugar from glycerol and 4.–sugar from fatty acids.

It might be suggested that the loss of water from the tissues on a fat diet, combined with a transport of muscle glycogen to the liver, would explain the seemingly absolute increase in glycogen in the body of the rat. The criticism may apply to the tissue analyses but it has no bearing on the liver results which in the fat-fed average 23.8 per cent heavier than the controls and in addition contain two to three times as much glycogen.

TABLE V

	No. Rats	Mg./gm. liver	Mg./gm. rat	Remarks
Control	15	2.98	148.0	All rats included
Fat-fed	15	10.49	172.0	All rats included
Fat-fed	11	12.89	180.3	Rats showing no liver glycogen deposits excluded from average.

Muscle glycogen does not appear to have been transported to the liver in large amounts. In group IIb muscle glycogen determinations were made. The average mg. per cent in the two groups is very similar, the controls averaging 1.85 and the fat-fed group 1.92. Individual values for the two groups are 2.35, 2.35, 1.45, 1.45, 1.71 and 2.86, 1.12, 2.22, 1.47, 1.96 and 2.12 respectively. It is quite clear, then, that most of the observed increase in sugar is an actual formation of sugar.

That protein may be the source of this glycogen is quite possible. A portion of the carbohydrate from protein normally oxidized may be stored in this condition of carbohydrate deprivation. This would tend to lower the R. Q. and might aid in explaining the few low quotients obtained.

Sugar formation from glycerol is a reasonably safe explanation for these glycogen deposits. But the smallness of the storage and its slow rate of formation make it appear somewhat unreasonable to assume that its source is mainly glycerol. The latter substance, if fed to a carbohydrate-poor animal, is quickly and largely converted to glycogen. Since, however, the feeding of the soaps of butter fat had no effect on liver glycogen, one must conclude that the presence of glycerol is somehow associated with this process.

Can it be explained on the basis of a slow transformation of fatty acids

to carbohydrate? The very slowness of the process might be taken to indicate this. A process of such magnitude would affect the R. Q. within the probable error of the determination. Even the taking of quotients during the period of maximum absorption, which presumably coincides with maximum conversion and storage, would be of little aid in separating out such a process. For example, rat 1, in group IIIb, stored 148 mg. of glycogen. If it be assumed that during a 1-1/2 hour metabolism period one gram of fat is absorbed, then about 7 mg. of glycogen would be stored in excess of that deposited and oxidized. This rat, 246 grams in weight, in 1-1/2 hours would use about 470 cc. of oxygen at R. Q. of 0.71. To form the 7 mg. of glycogen would require an extra 2.5 cc. of oxygen and the R. Q. would be lowered to 0.707, a figure well within the experimental error of the method. Such a process, then, would have a negligible effect on the quotient.

The calculation is in keeping with the R. Q.'s obtained. These average 0.707, 0.718, 0.720, 0.729, 0.732 for the five rats in groups II and III, with an average of 0.720. There was little or no depression of the quotient during absorption. Certainly the quotients found in association with the small deposition of glycogen do not indicate that conversion is occurring.

No explanation can be advanced for the higher quotients in group IIIb as compared with group IIb. The glycogen content of the controls in this series averages even lower than in group Ib, which would seem to rule out as a possible explanation the oxidation of preformed glycogen during fat absorption.

Using, however, the R. Q.'s in group IIIb as a basis for calculation, it is found (Table IV, column 9) that the sugar formed and oxidized plus that stored is 83 per cent of that derivable from non-fatty acid sources. In group IIb the average is 60 per cent. These figures are without doubt much too high for they are founded on the assumption that R. Q.'s exceeding 0.707 indicate sugar oxidation. The true figure lies somewhere between 0.707 and 0.720.

Summary

1. Twenty-four-hour starved rats when fed upon a diet of filtered butter fat for three to eight days exhibit a gradual formation of liver glycogen averaging three to four times the concentration in control animals. The total glycogen content of the fat-fed rats exceeds the controls by 22 per cent.

2. The amount of glycogen deposited is equivalent to only about 2 per cent of the ingested glycerol and probably has its source in this substance.

3. One rat fed the soaps of butter fat failed to show any increase in liver glycogen over the controls.

4. In groups Ib and IIb there is a depression of the urinary nitrogen excretion as compared with the control 24-hour starvation period.

5. The average for all respiratory quotients is 0.720. The individual figures, during fat absorption, do not require an explanation other than direct fat oxidation.

6. There is no evidence in the foregoing data to substantiate the hypothesis that fatty acids can be converted into carbohydrate.

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FOOD INTAKE IN PREGNANCY, LACTATION, AND REPRODUCTIVE REST IN THE HUMAN MOTHER*

By

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WITH a better understanding of the basic principles of nutrition it has become evident (1 to 12) that every comprehensive study of the growth and development of the child should include some knowledge of the nutritional status of the mother preceding and during the reproductive cycle. Mendenhall (13) states that

> "During pregnancy, proper prenatal hygiene and feeding is essential to the life and normal development of the unborn child. To count on the fact that the mother is a factor of safety in the nutrition of the young, and to nourish the child either at the expense of the pregnant or nursing mother is an unnecessary sacrifice of the woman and may at any time prove disastrous to the child."

Furthermore, she continues,

> "there is every reason to believe that ability to produce breast milk of a superior quality is to some extent dependent on the storage of material from the mother's food during the prenatal period, as well as on the supply of nutrients from the food she receives during lactation."

Though it is acknowledged that the proper kind and amount of food is essential to the mother during pregnancy and lactation if she is to maintain good health and at the same time properly nourish her baby before and after birth, the literature is practically barren of any extended studies on the effect of diet on human reproduction, and the feeding of women during this crucial period still remains on an empiric basis. Our present knowledge

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of maternal nutrition therefore falls short of adequate information for the mother, and, indeed, beyond the point where practical experience avails, it is impossible to teach her how to live so as to produce a nutritionally stable child and at the same time conserve her own body tissues.

Sherman (14) emphasizes the fact that "too much weight must not be attached to any of the so-called dietary standards" which are at best "only an indication, not a rule." In this connection he quotes Hart, McCollum, Steenbock and Humphrey, who say

> "We need more effort placed on the accumulation of information on the physiological behavior of feeding stuffs than on the attempts to bring out new mathematical expressions of feeding standards."

Many current dietary standards for human reproduction, as a review of the literature indicates, have been stated empirically. The importance of the problem justifies a more thorough study of the nutritional needs of women during the reproductive cycle. The development of standards must include a more quantitative statement of the nutritional demands for growth and maintenance upon which the additional requirements for reproduction are superimposed.

It has been emphasized in previous publications (3, 4, 11, 15, 16) from these laboratories that there are many internal and external factors operating during pregnancy and lactation to influence maternal and fetal physiological processes, the significance of which bears directly upon nutrition but the intensity of which is little understood. For this reason it has been pointed out (3) that

> "It is the accumulation and recording of many and diverse data on the same individual and on different individuals in various stages of gestation that will ultimately reveal the functions and physiological processes peculiar to the progression of the human reproductive cycle."

As a part of a series of studies on the metabolism of women during the reproductive cycle and the factors operating to influence human milk secretion, dietary studies of the quality and quantity of food chosen by women in their own homes during and after a reproductive cycle have been made. The present report, which is one of a series on the dietary requirements of women during the complete reproductive cycle, contains a record of the calorie, protein, fat, carbohydrate, calcium, and phosphorus intakes of three women, whose physiologic processes are known to be well adapted to the requirements of pregnancy and lactation (3, 11, 15) at intervals throughout a complete reproductive cycle.

Procedure

The women subjects of this investigation were multiparae with superior capacities to produce milk. They were housewives living in their own homes and doing most of their household work. Previous publications (3, 4, 11, 15, 16) have recorded data on some of the physiological processes of these women during the reproductive cycle and serve as a background for the consideration of the dietary observations reported herein. All of the milk of these women was expressed by hand. The milk their babies did not take was sold to the Mother's Milk Bureau of Detroit. Subjects VI, VII, and VIII had an average daily milk output of 3134, 2366, and 1420 cc., respectively.

The dietaries were entirely self-chosen and included the foods the women liked and believed to favor milk flow. The dietary observation periods occurred at approximately monthly intervals throughout two lactation cycles of fourteen months' duration and the intervening pregnancies for Subjects VI and VII and throughout one pregnancy and the subsequent fourteen-month period of lactation for Subject VIII.[1] All foods were weighed and prepared in the home in such a manner that the amount of each ingredient consumed could be determined accurately. Approved methods (17, 18) of calculations were used to determine the essential food components that were consumed in the dietary.

Experimental Data

The maternal metabolism seems to be stimulated in response to the increased requirements for intrauterine growth and the concurrent formation of maternal tissue reserves in preparation for parturition and lactation. Demands over and above these are made in the process of the elaboration of milk, and, furthermore, immediately following the cessation of milk flow, that is, in the post-lactation period, the maternal body undergoes a stage of recuperation from the extended physiological demands of reproduction and an augmented food intake is maintained. In some species of animals there is a general increase in food consumption occurring simultaneously with the progression of the reproductive cycle, except immediately preceding parturition, when a slight depression is usually noted. The food intake remains high in the early part of reproductive rest for the purpose of rebuilding the maternal tissues that suffered losses during pregnancy, parturition, and lactation.

The above facts are demonstrated in the experiments of John and Schick

[1] Subject VIII showed a persistent albuminuria with no other apparent indications of ill health.

(19), Slonaker (20), Wang (21), and Goss and Schmidt (22), who showed that rats on a satisfactory diet increased their food intake somewhat during pregnancy and approximately trebled it during lactation. Additional data substantiating these findings are presented in Table I. These show

TABLE I

PERCENTAGE INCREASE IN FOOD INTAKE OF RATS DURING PREGNANCY, LACTATION, AND POST-LACTATION ABOVE THE PRE-GESTATION INTAKE

Rat No.	Pregnancy	Lactation	Post-Lactation
416	46	167	
1194	31	87	
867	20	116	−10
529	44	145	46
870	6	111	12
530	−10	78	
530	46	189	78
529	21	86	32
529	0	75	
659	32	139	30
929	42	185	
757	− 4	66	11
753	6	53	28
656	49	125	43
803	23	70	0
754	3	66	
1124	3	54	− 5
531	26	130	
531	8	106	
416	23	136	− 2
416	49	173	
417	10	113	33
756	− 6	77	6
755	20	164	10
889	− 4	63	
Average	19	111	21

that the food ingested by rats in pregnancy, lactation, and post-lactation, as based upon the intake over the three weeks immediately preceding conception, increased on an average of 19 per cent in pregnancy, 111 per cent in lactation, and 21 per cent in post-lactation. The requirements for pregnancy and post-lactation were approximately the same but the demands for lactation were appreciably greater than for pregnancy or the post-lactation period.

It is believed that the human mother's ability to produce breast milk of a

satisfactory quality is to some extent dependent on the storage of material from her food during pregnancy, as well as on the supply of nutrients from the food she consumes during lactation. The literature at our disposal reveals no extended observations on the food consumption of individual women during pregnancy, lactation, and reproductive rest. Information of the actual food intake of the individual woman in different stages of the reproductive cycle is therefore pertinent to a better understanding of the dietary requirements of women at this time and likewise to the establishment of dietary standards for maternity.

Observations upon three women whose food records are given herein are in accord with the findings respecting the food intake of rats during the reproductive cycle. In Tables II, III, and IV are recorded for Subjects VI, VII, and VIII, respectively, the average daily intakes of protein, fat, carbohydrate, calories, calcium, and phosphorus over three or more successive days at frequent intervals during pregnancy and lactation. To aid in direct comparison of one subject with another, these data are computed on a basis of unit body weight and summarized in Table V.

The women of this investigation had experienced long and continuous reproductive cycles. At the beginning of this study Subjects VI and VII were in their third and second lactation periods, respectively, and Subject VIII in her third pregnancy. Subjects VI and VII were followed through two lactation periods with an intervening pregnancy and Subject VIII throughout a complete reproductive cycle and in reproductive rest. Subjects VI, VII, and VIII bore infants with birth weights of 8 pounds 10 ounces, 9 pounds 3 ounces, and 6 pounds 12 ounces, respectively, and all infants grew at the rate of approximately 4 ounces per week during the initial forty-five weeks of life, the first twenty-six weeks of which they were given little food other than their mother's milk. All three women lactated for fourteen months with a milk production in excess of that needed for the nourishment of their babies.

It is common knowledge that some women gain in body weight during the lactation period, while others lose. Subjects VI and VII lost in body weight during lactation, while Subject VIII gained. A detailed discussion of the qualitative and quantitative aspects of food consumption in relation to nitrogen metabolism and physiological behavior in milk secretion will appear in later publications. The liquid intake has already been discussed in relation to milk flow and body weight (15).

In all three cases there was an augmented food consumption in lactation. From pregnancy to lactation the average daily calorie intake of Subjects VI, VII, and VIII increased from 3300 to 4200, 2900 to 4500, and 2600 to

TABLE II
DISTRIBUTION OF FOOD ESSENTIALS IN THE SELF-SELECTED DIET OF SUBJECT VI

Height 64.8 inches

Date	Week of reproductive cycle	Days	Body weight (kilos.)	Av. Daily milk output (cc.)	Computed Average 24-hour Consumption						Nutritive ratio (1:)	Ca/P
					Cal.	Prot. (gm.)	Fat (gm.)	CHO (gm.)	Ca (gm.)	P (gm.)		
1927					Third Lactation (A)							
Dec.	60	10	63.1	1923	4600	172	193	534	2.92	3.43	5.6	0.85
1928					Fourth Pregnancy							
Mar.	20	4	74.7		3700	161	159	394	2.61	3.15	4.6	0.82
May	26	4	80.6	Birth	3000	126	137	326	1.93	2.40	5.0	0.80
June	30	4	84.2	Weight	3300	139	144	328	2.23	2.70	4.9	0.82
July	34	4	86.5	of	3100	129	134	357	1.76	2.33	5.0	0.75
Aug.	38	4	86.9	Infant	3200	110	122	406	1.90	2.21	6.1	0.86
Average for		20		8 lbs. 10 oz.	3300	133	139	343	2.09	2.56	5.1	0.81
1928					Fourth Lactation (B)							
Sept.	7	4	73.1	3213	3800	149	149	462	2.60	2.99	5.3	0.87
Oct.	10	6	70.8	3167	4000	139	189	437	2.56	2.81	6.2	0.91
Nov.	14	5	68.8	3530	4600	172	221	476	3.31	3.66	5.7	0.90
Dec.	19	7	67.2	3450	3600	145	153	423	1.97	2.49	5.3	0.79
1929												
Jan.	23	6	65.5	3547	4200	162	173	492	2.33	2.97	5.4	0.78
Feb.	27	4	64.7	3448	4600	177	191	543	3.28	3.61	5.5	0.91
Mar.	30	5	65.0	3534	4200	157	176	523	2.56	3.05	5.9	0.84
Apr.	34	6	65.0	3440	4400	159	202	507	2.83	3.25	6.0	0.87
May	38	4	64.7	3417	4800	174	219	544	3.09	3.48	4.5	0.89
June	42	4	64.0	3159	4000	137	195	431	2.41	2.72	6.3	0.89
July	46	1	64.7	2516	3200	160	122	370	2.70	3.19	4.1	0.85
Oct.	60	4	65.5	2856	4700	160	230	542	2.44	2.94	6.6	0.83
Average for		56		3134	4200	158	185	480	2.67	3.10	5.6	0.86
Average for		66			Third and Fourth Lactation 4200	169	186	484	2.69	3.12	5.7	0.86

Height 68.5 inches

TABLE III
DISTRIBUTION OF FOOD ESSENTIALS IN THE SELF-SELECTED DIETS OF SUBJECT VII

Date	Week of reproductive cycle	Days	Body weight (kilos.)	Av. Daily milk output (cc.)	Computed Average 24-hour Consumption						Nutritive ratio (1:)	Ca/P
					Cal.	Prot. (gm.)	Fat (gm.)	CHO (gm.)	Ca (gm.)	P (gm.)		
					Second Lactation (A)							
1927 Oct.	50	10	64.5	1197	4800	185	194	573	3.75	4.12	5.5	0.90
					Third Pregnancy							
1928 Feb.	14	4	66.3	Birth	3300	114	133	422	1.66	2.22	6.3	0.74
May	26	5	72.1	Weight	3000	120	122	336	1.79	2.31	5.1	0.77
June	30	4	73.1	of Infant	2800	125	122	307	1.70	2.44	4.7	0.70
July	34	4	75.0		2800	109	114	332	1.80	2.28	5.4	0.78
Aug.	38	4	75.6		2700	107	114	306	1.66	2.20	5.2	0.97
Average for		21		9 lbs. 3 oz.	2900	115	126	340	1.75	2.19	5.3	0.75
					Third Lactation (B)							
1928 Oct.	7	4	65.0	2683	4400	151	194	511	3.35	3.45	6.3	0.97
Dec.	13	4	68.0	2550	5600	216	182	754	3.16	3.84	5.4	0.82
1929 Jan.	18	3	66.5	2715	4600	148	135	713	2.99	3.71	6.9	0.81
Feb.	22	4	64.3	2444	3500	134	139	436	2.83	3.04	5.6	0.93
Mar.	25	4	63.4	2458	4600	167	197	527	3.16	3.70	5.8	0.85
Apr.	29	5	62.0	2485	4100	149	111	620	2.20	2.66	5.8	0.83
May	36	4	61.0	2506	5300	193	226	633	2.66	3.28	4.5	0.81
June	40	4	60.4	2284	4000	170	128	547	2.35	2.82	4.9	0.83
Nov.	62	3	61.8	1706	4600	139	168	683	1.73	2.48	7.7	0.70
Average for		35		2366	4500	163	164	603	2.71	3.22	5.9	0.84
					Second and Third Lactation							
Average for		45			4500	155	165	610	2.82	3.31	5.8	0.85
					Reproductive Rest							
1930 Feb.	11*	3	66.8		3500	97	139	463	1.10	1.64	8.0	0.67
Oct.	41*	9	65.8		4000	122	139	558	1.44	1.80	6.4	0.80

* Weeks after cessation of milk flow.

TABLE IV.
DISTRIBUTION OF FOOD ESSENTIALS IN THE SELF-SELECTED DIETS OF SUBJECT VIII

Height 62.5 inches

Date	Week of reproductive cycle	Days	Body weight (kilos.)	Av. daily milk output (cc.)	Average Computed 24-hour Consumption						Nutritive Ratio (1:)	Ca/P
					Cal.	Prot.	Fat (gm.)	CHO (gm.)	Ca (gm.)	P (gm.)		
1928					Third Pregnancy							
May	30	4	79.2	Birth	2700	101	126	302	2.41	1.94	5.8	1.24
June	34	4	79.8	Weight of	2400	78	98	308	1.53	1.46	6.8	1.05
Average for		8		6 lbs. 12 oz. Infant	2600	90	112	305	1.97	1.70	6.3	1.15
1928					Third Lactation							
Aug.	7	4	70.4	1490	3800	165	195	356	3.71	3.62	4.8	1.12
Oct.	14	6	75.0	1468	3600	127	159	444	2.79	2.81	6.3	0.99
Nov.	18	8	76.7	1545	3700	141	128	443	2.53	2.80	5.2	0.90
Dec.	23	8	78.5	1550	4300	165	174	509	3.04	3.21	5.5	0.95
1929												
Jan.	27	4	80.8	1654	4000	160	211	410	3.43	3.40	5.5	1.38
Feb.	30	9	82.2	1449	4300	166	175	507	3.13	3.36	5.4	0.93
Mar.	34	7	82.1	1558	3800	149	170	417	2.93	3.01	5.4	0.97
Apr.	38	8	82.2	1515	3300	141	138	382	2.76	2.76	4.9	1.00
May	43	4	81.5	1518	4200	154	189	415	3.24	3.63	5.3	0.86
June	47	4	81.7	1418	3900	138	164	433	3.09	3.20	5.8	0.97
Sept.	63	4	81.4	1323	3900	155	179	420	3.40	3.31	5.3	1.03
Oct.	65	3	81.8	952	3300	147	150	347	2.95	3.06	4.6	0.96
Average for		59		1420	3800	151	170	425	3.08	3.18	5.3	1.01
1930					Reproductive Rest							
Jan.	13*	4	81.4		2500	85	92	326	1.47	1.54	6.3	0.95
Sept.	45*	9	80.8		2900	115	112	339	1.65	1.93	5.7	0.85

* Weeks after the cessation of milk flow.

TABLE V
AVERAGE DAILY FOOD INTAKE PER KILOGRAM OF BODY WEIGHT

Subject	VI			VII				VIII			
Reproductive Stage	Pregnancy	Lactation	Increase[1] in lactation, per cent	Resting	Pregnancy	Lactation	Increase[1] in lactation, per cent	Resting	Pregnancy	Lactation	Increase** in lactation, per cent
No. of Days Observed*	20	64		12	21	45		13	8	59	
Calories	41	65	60	59	41	70	70	34	33	49	49
Protein, gm.	1.7	2.4	45	1.8	1.6	2.6	59	1.3	1.1	1.8	59
Fat, gm.	1.7	2.9	71	2.1	1.7	2.6	52	1.3	1.4	2.1	49
Carbohydrates, gm.	4.4	7.4	69	8.1	4.8	9.4	99	4.2	3.8	5.2	36
Calcium, gm.	0.026	0.042	62	0.021	0.024	0.045	88	0.020	0.025	0.045	80
Phosphorus, gm.	0.030	0.047	57	0.027	0.031	0.052	68	0.024	0.022	0.043	95

* Each period of observation was three or more consecutive days in length.
** Per cent increase over pregnancy intake.

3800, respectively. Similarly, the protein intake increased from 133 to 158, 115 to 155, and 90 to 151 grams. There was also an increase in the average ingestion of other food essentials from pregnancy to lactation.

The per cent increase in the intake of various food essentials from pregnancy to lactation has been calculated on a kilogram body weight basis and recorded in Table VI. The average percentage for the three women

TABLE VI
PER CENT INCREASE IN FOOD ESSENTIALS IN LACTATION OVER THOSE CHOSEN IN PREGNANCY
(per kilo body weight)

Subject	VI	VII	VIII	Average
Calories	60	70	49	60
Protein, gm.	45	59	59	54
Fat, gm.	71	52	49	57
Carbohydrate, gm.	69	99	36	68
Calcium, gm.	62	88	80	77
Phosphorus, gm.	57	68	95	73

TABLE VII
VARIATIONS IN QUANTITY OF FOOD CHOSEN BY TWO WOMEN DURING TEN CONSECUTIVE DAYS OF MATURE LACTATION

Subject		Protein (gm.)	Fats (gm.)	Carbohydrate (gm.)	Calcium (gm.)	Phosphorus (gm.)	Calories
VI	Maximum	221	247	715	3.82	4.28	5500
	Minimum	147	134	416	2.18	2.81	3500
	Average	172	193	534	2.92	3.43	4600
VII	Maximum	240	279	719	5.30	5.33	5800
	Minimum	121	125	381	2.03	2.47	3100
	Average	185	194	573	3.75	4.12	4800

demonstrates the following increases: Calcium, 77 per cent; phosphorus, 73 per cent; carbohydrate, 68 per cent; energy, 60 per cent; fat, 57 per cent; and protein, 54 per cent. These data show that, as compared with the food intakes during pregnancy, lactation brought about an increased consumption of all dietary essentials.

There is a slight curtailment of food consumption near the close of pregnancy (Tables II, III, IV), as has been shown in the case of some animals (20). There was less activity as gravidity progressed, even though the women assumed the major responsibility of their housework and the care of

their families. However, there was no constant drop in food intake near the end of lactation. On the contrary, observations on Subjects VII and VIII during reproductive rest, three and eleven months after the cessation of milk flow, showed that the food consumption was practically the same as during pregnancy and that the augmentation of requirements in pregnancy and post-lactation were about the same. The gravid woman instinctively refrains from excessive bodily activity, and this inactivity, calling ordinarily for less than the average food intake, paralleled by the augmented food intake during pregnancy, doubtless obscures an increased demand for nourishment during the last part of pregnancy. Since these subjects had been continuously under the demands of the reproductive cycle for a considerable period of time, with long and intensive lactation periods, it is difficult to predict the recuperative demands in post-lactation and reproductive rest. Lactation in all three women increased the food demands for all dietary constituents approximately 60 per cent over and above those for pregnancy. Lactation, then, is truly the drain of pregnancy in a more intense degree (23).

The amount of food chosen and ingested by each woman varied greatly from day to day. The figures given in Tables II, III, and IV are averages for three or more successive days. The marked daily fluctuations in intake of the various food essentials in ten consecutive days of mature lactation, for Subjects VI and VII, are illustrated in Table VII. The maximum intake was at times more than twice the minimum. Thus the calorie intake for Subjects VI and VII varies from 3500 to 5500 and 3100 to 5800, respectively; similarly, protein ranged from 147 to 221 and 121 to 240 grams, respectively. The greatest variations occurred in the mineral constituents of the diet. Thus, the calcium intake varied from 2.18 to 3.82 and 2.03 to 5.30 grams and the phosphorus fluctuated from 2.81 to 4.28 and 2.47 to 5.33 grams, respectively, for Subjects VI and VII. As the maxima and minima indicate, the variations in the amounts of the different food components do not coincide, showing that the proportions of the different food components, as well as the amount of food, varied considerably from day to day.

From the data presented, the season of the year cannot be said to be a dominant factor in food consumption, since all three women had access to fresh fruits and vegetables throughout the year.

Summary

1. The food ingested by rats in pregnancy, lactation, and post-lactation, as based upon the intake over the three weeks immediately preceding con-

ception, increased on an average of 19 per cent in pregnancy, 111 per cent in lactation, and 21 per cent in post-lactation.

2. This paper records the intake of energy, protein, fat, carbohydrate, calcium, and phosphorus at intervals throughout a complete reproductive cycle for three women whose physiological processes are known to be adapted to the requirements of pregnancy and lactation.

3. The food requirements for pregnancy and for recuperation in post-lactation are of approximately the same magnitude.

4. There is a curtailment of food consumption near the close of pregnancy.

5. Lactation increases the food demands approximately 60 per cent over and above those of pregnancy.

6. There are marked daily fluctuations in food intake during lactation, the maximum being at times more than twice the minimum.

Note. An interesting report by Sandiford, Wheeler, and Boothby (24), giving the food intake of one woman from the 13th to 40th week of pregnancy, appeared after the completion of the present paper.

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GLYCOGEN AND FAT FORMATION IN RATS

V. CARBOHYDRATE-FREE DIETS

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THE experiments on carbohydrate-free diets were performed during the months of June and July, 1930. Fifty rats of the Long-Evans strain were taken at weaning time (3 weeks), and placed on McCollum's Stock Diet I for an observation period, during which time (1½ to 3 weeks) the gain in weight, daily food intake, and water consumption were measured. For the males, the mean initial weight was 46.8 grams, (range 36 to 69), the mean observation period 15.1 days, mean daily gain 4.1 grams, and the mean final weight 108.7 grams (range 83 to 145). For the females, the values found were 43.4 grams (range 32 to 59), 14.3 days, 3.0 grams, 86.9 grams (range 75 to 102), respectively. At the end of the observation period, 10 rats (6 males and 4 females) were placed on each of the 5 special diets and records kept as before. (Two rats developed infections, and were eliminated). At the end of 21 days on the special diets, the rats were killed, and liver glycogen (1) and liver fat determined.

The special diets contained lactose-free casein, lard and McCollum's "salt-mixture 185." Each diet was supplemented by daily vitamin feeding, as before described (1). The diets were composed as follows:

1.—87.5 per cent of the total caloric value in the form of lard, 12.5 per cent in the form of casein, the mixture having a caloric value of 7.07 per gram; 2.—lard 75.0, casein 25.0, caloric value of mixture 6.19; 3.—lard 50.0, casein 50.0, caloric value of mixture 4.95; 4.— lard 25, casein 75, caloric value of mixture 4.12; and 5.—lard 12.5, casein 87.5, caloric value of mixture 3.80 (Salt mixture 185–1 gram per 100 calories in each diet). The caloric value was reckoned as follows: by analysis, the lactose-free casein was found to contain 14.37 per cent of nitrogen. The theoretical value, according to Mathews, is 15.7 per cent. The casein as used, therefore contained 91.5 per cent casein, instead of 100 per cent. In making up Diet 5, for example, 2390.7 grams of casein, 138.9 grams of lard, and 100 grams of salt mixture 185, were used. $2390.7 \times .915 \times 4 = 8750$ cal., or 87.5 per cent of total. $138.9 \times 9 = 1250$ cal. or 12.5 per cent of total. The

total weight was 2629.6 grams, the caloric value 10,000; therefore each gram had a caloric value of 3.80.

Results

Preliminary Period

The mean daily caloric intake on the stock diet (3.88 calories per gram), daily water intake, daily gain in weight, and final weight at the end of the observation period, are shown in Table I. (The water intake was determined by subtracting the amount left in the bottle after a definite time from the original amount.)

Table I
Observation Period

Rats placed later on diet	Mean daily caloric intake	Mean daily water intake cc.	Mean daily gain in weight gms.	Mean final weight gms.
1	37.64	12.3	4.2	91.5
2	34.84	11.2	4.0	90.7
3	34.49	10.4	3.9	105.4
4	32.20	10.9	3.3	105.4
5	32.98	11.1	3.5	110.8

The results indicated that the rats were in good condition, as far as food and water consumption and gain in weight were concerned, during the observation period, and at the time they were placed on the special diets.

Results on Special Diets

When the rats were changed from the stock diet to the special diet, some adjustment was necessary; consequently, it seems best to present the results for each week separately. The results for water intake are shown in Table II.

Table II
Mean Daily Water Intake (cc.)

Diet	First week	Second week	Third week	Mean for entire period
1	7.5	8.1	8.3	8.0
2	11.6	13.3	13.5	12.8
3	11.4	13.5	15.6	13.5
4	8.7	13.3	18.0	13.3
5	10.9	15.3	18.5	14.9

It will be noticed that after adjustment (first week), the water intake increased in general, with increase in per cent of protein in the diet. On Diet 1, the water intake decreased 34.9 per cent below that on the stock diet, while it increased 14.3, 29.8, 22.0, and 34.2 per cent on Diets 2, 3, 4 and 5, respectively.

The mean daily caloric intake for each week is shown in Table III.

TABLE III
MEAN DAILY CALORIC INTAKE

Diet	First week	Second week	Third week	Mean for entire period
1	38.32	45.38	41.99	41.90
2	38.25	42.83	43.82	41.63
3	25.24	30.39	31.38	29.00
4	13.60	19.15	21.26	19.50
5	13.11	16.99	20.63	18.79

The caloric intake on Diet 1 increased 11.3 per cent above that on the stock diet; on Diet 2, the increase was 19.5 per cent. On Diets 3, 4, and 5, the caloric intake decreased 15.9, 39.4, and 43.0 per cent, respectively. Either the rats did not like the diets containing more than 25 per cent of the total caloric value in the form of casein, or less food was required.

Such a variation in caloric intake led to a similar variation in weight. During the first week, the most marked discrepancies in weight occurred, as would be expected. There were 5 types of response found. These were: 1.—straight gain in weight (9 animals on Diet 1, 9 on Diet 2, and 1 on Diet 3); 2.—some loss, then gain, until the weight at the end of the week was above that at the beginning (1 animal on Diet 1, 1 on Diet 2, 7 on Diet 3 and 2 on Diet 4); 3.—some loss, followed by some gain, the weight at the end of the week being still below that at the beginning (2 on Diet 3, 3 on Diet 4, and 6 on Diet 5); 4.—some loss, with the same amount of gain, the weight at the end being equal to that at the beginning (1 on Diet 4 and 1 on Diet 5); 5.—some loss, no gain, the lowest point in the weight curve occurring at the end of the first week (3 on Diet 4 and 2 on Diet 5). No losses occurred during the second and third weeks.

The mean gains in weight for all the rats on each diet are presented in Table IV.

In order to determine the relationship between the caloric intake and the gain in weight, the coefficients of correlation (ρ) were calculated. Table V presents the results.

With the exception of the third week on Diet 1, the relationship between the caloric intake and gain in weight is significant, regardless of the diet.

TABLE IV
MEAN GAIN IN WEIGHT

Diet	First week	Second week	Third week	Entire Period (21 days)
1	18.1	24.7	22.8	65.6
2	27.5	27.9	25.3	80.7
3	7.4	18.6	16.5	42.5
4	−8.3	12.7	12.1	16.5
5	−8.6	10.5	11.1	13.0

The absolute and the relative (expressed as per cent of body weight) liver weights, are shown in Table VI. The relative liver weights were first

TABLE V
CORRELATION BETWEEN CALORIC INTAKE AND GAIN IN WEIGHT

Diet	First week Correl. Coeff.	$\frac{\rho}{\text{P.E.}}$	Second week Correl. Coeff.	$\frac{\rho}{\text{P.E.}}$	Third week Correl. Coeff.	$\frac{\rho}{\text{P.E.}}$
1	+.760±.090	8.4	+.744±0.095	7.8	+.329±0.19	1.7
2	+.702±.114	6.2	+.862±0.058	14.9	+.873±0.053	16.5
3	+.757±.091	8.3	+.532±0.15	3.5	+.788±0.081	9.7
4	+.757±.102	7.4	+.677±0.13	5.2	+.692±0.12	5.8
5	+.631±.143	4.4	+.792±0.089	8.9	+.627±0.14	4.5

determined for the two sexes separately. Since no statistically significant difference was found between the two sexes on any one of the diets, the mean weights presented include both sexes. The differences between the

TABLE VI
LIVER WEIGHTS

Diet	Mean absolute liver weights gms.	Mean relative liver weights per cent	Difference in relative weights—Diet 2 as subtrahend	$\frac{\text{Difference}}{\text{P.E. diff.}}$
1	7.48±.32	4.729±.126	+ .22±.15	1.5
2	7.77±.32	4.505±.087	—	—
3	6.98±.26	4.680±.097	+ .18±.13	1.3
4	6.82±.35	5.565±.170	+1.06±.19	5.6
5	7.14±.30	5.742±.074	+1.24±.11	11.3

relative liver weights on the various diets are also presented in Table VI. Diet 2 (25 per cent of the total caloric value in the form of protein) is used throughout as the subtrahend.

It will be noted that the liver is significantly heavier, in comparison to body weight, on the diets containing 75 and 87.5 per cent of the total caloric value in the form of protein.

The per cent of liver glycogen and per cent of liver lipid are shown in Table VII.

TABLE VII

Diet	Liver Glycogen (per cent)	Liver Lipid (per cent)
1	1.64±.10	12.80±.77
2	3.13±.17	5.62±.44
3	2.73±.10	4.13±.10
4	1.87±.14	3.05±.10
5	2.44±.10	2.94±.06

The optimum diet for glycogen formation was the one containing 75 per cent of the total caloric value in the form of lard and 25 in the form of casein (diet 2). However, even with 87.5 per cent of lard (diet 1), considerable glycogen was formed. The liver lipid content varied as the percentage of lard in the diet.

During the fall months of 1930, an entirely different type of experiment was used. Adult discard rats were used. The animals were fasted 48 hours, and then fed the test food for 24 hours and then killed. In order to establish standards, 9 female and 3 male rats were killed at the end of the 48 hour fast. The results are shown in Table VIII.

TABLE VIII
FASTING RATS (48 hrs.)

	Final weight (gms.)	Liver wt. (gms.)	Liver wt. as per cent of body wt.	Liver glycogen (per cent)	Liver lipid (per cent)
Females	194.3	4.620	2.397	.137	5.64
Males	303.0	7.519	2.477	.513	4.34

The most striking difference is in the per cent of liver glycogen. The difference is 0.376 per cent.

After determining the fasting glycogen level, rats were starved 48 hours,

then fed such test foods as stock diet, sucrose, lard, casein, and casein plus 20 per cent lard, for 24 hours, and then killed. The results are presented in Table IX.

TABLE IX

RATS STARVED FOR 48 HOURS, THEN FED 24 HOURS

Test Food		Final wt. (gms.)	Liver wt. (gms.)	Liver as per cent of body wt.	Liver Glycogen (per cent)	Liver lipid (per cent)
Stock	3 F.	194.2	7.444	3.87	5.20	3.11
Sucrose	3 F.	180.5	7.622	4.22	7.82	5.36
Lard	4 F.	178.2	5.295	2.96	.202	10.53
Lard	8 M.	260.4	6.446	2.56	.715	8.33
Casein	3 F.	173.3	5.628	3.26	1.95	3.53
Casein plus 20% lard	1 F.	176.0	6.974	3.96	1.88	4.64
Casein plus 20% lard	5 M.	262.4	9.061	3.48	1.81	3.51

Attempts were made to use the fatty acids from lard and their sodium soap as test foods. The rats ate such small amounts, if any, that the results are really those of a three-day fast. They ate glycerol, however, and formed glycogen, as will be shown. A few experiments were done on forced feeding. Oleic acid was given to one, and glycerol to several. The results are presented in Table X.

TABLE X

Test Food		Final wt. (gms.)	Liver wt. (gms.)	Liver as per cent of body wt.	Liver glycogen (per cent)	Liver lipid (per cent)
Sodium soap	3 M.	244.0	5.482	2.24	.190	9.40
Sodium soap	2 F.	179.5	4.037	2.26	.000	9.92
Fatty acids	2 M.	314.5	6.492	2.07	.260	11.14
Fatty acids	2 F.	206.0	4.515	2.22	.052	12.18
Oleic acid forced fed	1 M.	282.5	6.785	2.40	.207	4.61
Glycerol	7 M.	269.8	7.194	2.66	3.37	6.24
Glycerol	10 F.	213.1	5.898	2.78	2.63	7.37

I am unable to account for the sexual difference evident in the glycogen in the fasting rats, in those fed lard, sodium soap, fatty acid, or glycerol.

Summary and Conclusions

1. Young rats of the Long-Evans strain were fed test diets of lard, lactose-free casein and salt mixture 185 for 3 weeks, after a preliminary observation period. The composition of the diets was 1.—87.5 per cent of the total caloric value from lard and 12.5 per cent from casein; 2.—75.0 lard and 25.0 casein; 3.—50 lard and 50 casein; 4.—25 lard and 75 casein; 5.—12.5 lard and 87.5 casein.

2. The water intake was determined for each group on the stock diet, during the preliminary observation period and on the special diets. On diet 1, the water intake decreased 34.9 per cent; while it increased 14.3, 29.8, 22.0, and 34.2 per cent, on diets 2, 3, 4 and 5 respectively.

3. On diets 1 and 2, the caloric intake, as compared with the stock diet, increased 11.3 and 19.5 per cent respectively. On diets 3, 4 and 5 the caloric intake decreased 15.9, 39.4, and 43.0 per cent respectively.

4. The rats on diets 1, 2 and 3 gained steadily in weight, the mean total gain for the 21 days being 65.6, 80.7 and 42.5 gms. respectively. Those on diets 4 and 5 lost weight during the first week, but gained slowly during the second and third weeks. The mean total gain for the 21 days was 16.5 gms. for the rats on diet 4, and 13.0 gms. for those on diet 5.

5. There was a significant correlation between caloric intake and gain in weight, except in the rats on diet 1, during the third week of the test period.

6. The liver, in relation to the body weight, was significantly heavier in the rats on diets 4 and 5, than in those on the other diets. The livers of the rats on diets 1, 2 and 3 did not differ significantly from each other in relative weights.

7. The percentage of liver glycogen was 1.64, 3.13, 2.73, 1.87, 2.44 on diets 1,2, 3, 4 and 5, respectively. The glycogen formed on the first 2 diets may be partly from the casein and partly from the glycerol of the lard, as shown by later experiments.

8. The liver lipid content was 12.80, 5.62, 4.13, 3.05, and 2.94 per cent on diets 1, 2, 3, 4 and 5, respectively.

9. In later experiments, adult discard rats were fasted for 48 hours, then fed test food, during the last 24 hours before killing. The glycogen percentage dropped to 0.137 in female rats and 0.513 in male rats, on fasting 48 hours. Glycogen was readily formed by feeding the stock diet, sucrose, casein, or glycerol. Only a small amount was formed from lard. The experiments in which sodium soap and fatty acids were used as test foods were unsatisfactory, as the rats could not be induced to eat any amount. In

the one rat fed (forced) oleic acid, the glycogen content dropped below the fasting level.

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DISTRIBUTION OF MANGANESE IN FOODS*

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WITHIN the last few years it has been observed that a number of elements, although frequently occurring in only minute amounts, are universally present in plant and animal materials. Their universal occurrence can hardly be a matter of chance and, consequently, numerous investigators have attempted to ascertain what functions these elements perform in plants and animals. One of these elements, manganese, has received considerable attention from the standpoint of both plant and animal nutrition.

In 1913 Osborne and Mendel (1) observed that better growth of rats fed on "artificial protein-free milk" was secured when traces of iodine, fluorine, aluminum, and manganese were included in the ration. Daniels and Hutton (2), in 1925, reported that rats on a milk diet are able to produce five generations of normal young if small amounts of manganese, fluorine, and aluminum are added along with sodium silicate. The beneficial effects noted in these experiments, however, could hardly be attributed with certainty to the presence of manganese, since this element was only one of several added. Other investigations, especially in recent years, have been made in which manganese alone was added to various rations, some of which probably were inadequate from standpoints other than manganese deficiency. Levine and Sohm (3) reported that manganese produced a favorable effect upon rats as indicated by their appearance and activity as well as by a more rapid growth of their offspring. Richet, Gardner, and Goodbody (4) demonstrated that small amounts of manganese when administered to dogs every three days enhanced growth, whereas the reverse was true if the element was given more frequently. That manganese exerts a favorable effect upon the growth of mice and rats has been observed by Bertrand and Nakamura (5) and McHargue (6) respectively. McCarrison (7) likewise found that the element accelerated growth if given to the extent of 0.009 mg. per rat daily, but if the intake of manganese was increased

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to 0.56 mg. a definite retardation of growth followed. That the element is stored throughout life, but especially prior to maturity, and that the rate of storage can be markedly increased by the addition of manganese to either a complete or incomplete ration has been demonstrated by Skinner, Peterson and Steenbock (8).

With the discovery by Hart, Steenbock, and associates (9) that copper functions in hemoglobin formation, a new field of investigation was opened for the study of manganese as well as of various other elements. Titus and Hughes (10, 11) and Beard and Myers (12, 13) reported that manganese, like copper, is an active agent in the regeneration of hemoglobin, but the work of Hart, Steenbock, and associates (14, 15), Krauss (16), and Lewis and associates (17) throws the weight of experimental evidence against this conclusion.

Regardless of the final conclusion concerning the function of manganese in hemoglobin regeneration, most investigators are agreed that the element does play some important rôle in the animal organism. Therefore, in order that a sufficient amount of the element may be included in the human diet, a knowledge of its occurrence in various food materials is highly desirable. A previous publication from this laboratory (18) gave the manganese content of a number of our common food materials. More recently several investigators (19, 20, 21, 22) have contributed to the knowledge of the occurrence of this element in foods.

In this paper are presented the data for 83 food materials, the greater number of which have not been analyzed previously for manganese. The data given herein, together with those in a previous publication, have been combined so as to summarize concisely the occurrence of manganese in the common classes of foodstuffs.

Experimental

Since the method of preparation of the samples has been given in a previous publication (23), it need not be described here. The manganese determinations were run by the periodate method in which, with one exception, phosphoric acid was used for extraction of the ash. The procedure given by Davidson and Capen (24) was followed in the analysis of samples containing 0.1 mg. or more of manganese, which included mainly the vegetable materials. Foodstuffs containing very small amounts of the element were analyzed according to the modification used by the authors (25) in the determination of manganese in animal materials. The high chloride content of olives did not permit a determination of manganese by either of these procedures. Instead, the ash was extracted with dilute H_2SO_4, and

after the addition of 5 cc. of concentrated HNO_3 the chlorine was removed by evaporating until SO_3 fumes came off. To insure complete removal of the chlorine this step was repeated twice. The solution was then oxidized to permanganate and the color compared with the standard in the usual manner. In most instances, triplicate determinations, one of which involved the recovery of a known amount of added manganese, were made on each sample. If a recovery of 90 per cent or better was not obtained, the analysis was repeated.

Since it was the aim of this investigation to supplement the data previously reported, duplication of analyses was avoided. In two instances, however, it seemed desirable to check some of the results obtained in the previous investigation. In the former publication certain materials, *e.g.*, fish, were reported to contain either no manganese or only traces of the element. With a more sensitive method available, it seemed probable that the actual amounts of manganese in these materials could be detected and, accordingly, some of the samples have been reanalyzed. At the other extreme was a sample of blueberries which appeared unusually high in manganese. The original sample was reanalyzed and on more thorough oxidation was found to be even higher in manganese (209 mg. per kg., dry basis) than was previously reported (122.4 mg.). A new sample was therefore procured and was found to be still higher (314.9 mg. per kg.). From these results it appears that blueberries are normally high in manganese.

In Table I are given the moisture and manganese content, calculated on both the fresh and dry basis, of 83 representative food materials. It will be noted that in no case was the manganese concentration too low to warrant a numerical value, thus substantiating our belief that the more sensitive method would permit detection of the minute amounts present. On the fresh basis the concentration of manganese ranged from 0.028 mg. per liter of milk to 49.9 mg. per kilo. of bran flakes. Of the total number of foodstuffs analyzed, thirty-six were found to have a concentration of less than 1 mg., twenty-five from 1 to 5 mg., and only twenty-two more than 5 mg. of manganese per kilo of fresh material. On the dry basis the manganese content varied from 0.091 mg. in bacon to an exceptionally high concentration of 314.9 mg. per kilo. in blueberries. The six samples of milk varied from 0.017 to 0.048 mg. of manganese per liter. Three of these milks were produced by groups of the University herd on three different rations and three samples were obtained from herds in other states. Variations in manganese were less extensive among milks from the groups of the University herd than among milks from herds in other states.

TABLE I
MANGANESE CONTENT OF FOODS (EDIBLE PORTION)

Food*	Moisture	Manganese Content	
		Dry basis (100°)	Fresh material
	per cent	mg. per kg.	mg. per kg.
Almonds	3.9	20.2	19.4
Artichoke	84.4	23.2	3.6
Bacon	13.7	0.091	0.078
Beans, string (2)	91.4	28.2	2.4
Beef, casings	81.1	1.3	0.25
Beef, lung	80.3	2.7	0.52
Beef, pancreas	80.0	3.3	0.65
Beefsteak, T-bone	74.5	0.65	0.16
Blueberries	85.9	314.9	44.4
Brains, calf	80.7	2.3	0.44
Bran flakes	6.5	53.4	49.9
Brazil nuts	4.3	9.6	9.2
Bread, white	35.0	4.7	3.1
Butter	13.0	0.45	0.39
Cantaloupe	94.6	7.7	0.42
Cauliflower	93.0	25.2	1.7
Celery cabbage	95.0	23.3	1.2
Cheese, American	31.6	0.77	0.53
Cheese, cottage	75.0	2.1	0.53
Cheese, Swiss	37.5	3.1	1.6
Chestnuts, Italian	34.5	56.0	36.7
Chocolate, bitter	5.0	32.2	30.5
Cocoa	4.5	37.0	35.3
Coconut	46.4	25.0	13.1
Corn, sweet	68.0	4.7	1.5
Corn flakes	6.2	0.52	0.49
Corn-meal, white, prepared	6.2	1.6	1.5
Corn-meal, yellow, prepared	6.0	3.1	2.8
Cranberries	88.1	24.9	3.0
Cream of Wheat	7.4	4.8	4.5
Cucumbers	96.9	48.4	1.5
Dandelion greens	82.4	19.2	3.4
Eggplant	92.2	14.6	1.1
Eggs	72.8	1.1	0.30
Egg yolk	49.5	2.3	1.1
Endive	93.6	35.0	2.2
Filberts	2.0	42.8	41.7
Fish and sea foods			
Cod	80.7	0.63	0.12
Crab	77.1	1.3	0.30
Lobster	84.3	2.5	0.39
Mackerel	72.6	0.87	0.24
Oyster	90.0	20.8	2.1
Red snapper	79.2	0.63	0.13
Shrimp	71.0	1.0	0.29

TABLE I (*continued*)

Food*	Moisture	Manganese Content	
		Dry basis (100°)	Fresh material
	per cent	mg. per kg.	mg. per kg.
Flour, buckwheat	13.8	24.2	20.9
Flour, Graham	12.7	49.4	42.8
Flour, patent	12.0	4.5	4.0
Flour, rye	6.4	20.7	19.4
Hominy	9.7	1.2	1.1
Honey	12.6	0.36	0.31
Lamb chops	69.2	1.2	0.37
Lemons	88.9	3.6	0.40
Lettuce, leaf	93.8	167.0	10.4
Milk, whole			
Maximum		0.37	0.048**
Minimum		0.13	0.017**
Average (6)	87.5	0.22	0.028**
Milk, colostrum	85.1	0.28	0.044**
Milk, whole, powder	5.0	0.19	0.18
Molasses	25.7	5.5	4.2
Mushrooms	88.1	6.9	0.82
Oatmeal	9.9	31.0	27.9
Olives, green, canned	77.0	2.4	0.55
Oyster plant	72.0	12.4	3.5
Peas, split	9.5	30.7	27.7
Pecans	2.3	35.7	34.8
Peppers, green	92.5	19.1	1.4
Plums	88.0	6.2	0.74
Pork chops	63.1	1.6	0.59
Poultry			
Duck	43.7	0.58	0.33
Goose	57.0	1.2	0.52
Turkey, dark meat	72.2	1.6	0.45
Turkey, light meat	72.1	1.1	0.31
Puffed Rice	10.9	8.4	7.3
Puffed Wheat	8.6	30.2	27.2
Pumpkin	91.7	4.5	0.37
Radishes	96.2	13.7	0.52
Raspberries	88.9	45.9	5.1
Rhubarb	95.3	30.6	1.5
Rice, polished	13.3	12.9	10.8
Rutabagas	86.6	9.9	1.3
Shredded Wheat	8.1	25.9	23.9
Tomatoes	94.2	24.0	1.4
Turnips	92.0	5.4	0.43
Veal chops	78.5	1.3	0.28
Watercress (2)	92.2	69.1	5.4

* When more than one sample was analyzed, the number of samples is indicated by the figure in parentheses.

** mg. per liter.

A survey of the concentration of the element in classes of food materials is obtained from Table II. In this table are listed 12 major classes of foodstuffs, arranged in descending order of their manganese content, together with the minimum, maximum, and average for each group. This table, as mentioned above, has been compiled from the data in Table I and those in the previous publication (18) wherever numerical values were obtained for the manganese concentrations.

TABLE II

GROUPS OF PRINCIPAL FOODSTUFFS ARRANGED IN DESCENDING ORDER OF MANGANESE CONTENT—FRESH BASIS (EDIBLE PORTION)

Class of food	Number of samples	Manganese		
		Minimum	Maximum	Average
		mg. per kg.	mg. per kg.	mg. per kg.
Nuts	10	6.3	41.7	22.7
Cereals and their products	23	0.49	91.1	20.2
Dried legume seeds	4	10.7	27.7	20.0
Green leafy vegetables	18	0.76	12.6	4.5
Dried fruits	7	1.5	6.7	3.3
Roots, tubers, and stalks	12	0.35	9.2	2.1
Fresh fruits, excluding blueberries	25	0.18	10.7	2.0
Fresh fruits, including blueberries	26	0.18	44.4	3.7
Non-leafy vegetables	5	0.82	2.4	1.5
Animal tissue	13	0.078	3.8	1.0
Poultry and poultry products	6	0.30	1.1	0.50
Dairy products	7	0.028	1.6	0.47
Fish and sea foods, excluding oysters	6	0.12	0.39	0.25
Fish and sea foods, including oysters	7	0.12	2.2	0.51

Since the first three classes of foodstuffs listed in Table II, nuts, cereals, and dried legume seeds, are mainly dry matter, the manganese content is found to be correspondingly high. Leafy vegetables, which rank fourth in a comparison of materials in their native state, easily lead if the comparison is made on the dry basis. Since blueberries contain such an enormous amount of the element, the average for fresh fruits has been given both with and without the blueberry value. The average exclusive of blueberries is much more nearly representative of this class of foodstuffs and ranks the group in seventh place. The last three groups, poultry and poultry products, dairy products, and fish and sea foods are seen to be very low in manganese; all average about 0.5 mg. per kilo. of fresh material. In the group of fish and sea foods is found one material, oysters, which is much higher in manganese than the other members of the group. The average

manganese concentration of animal tissue (1 mg. per kg.) is doubtless greater than the average for meat actually consumed in the human diet since liver, a material containing a relatively large amount of the element, represents almost one-fourth of the samples analyzed, whereas it represents a much lower proportion of the meat consumed by an individual.

Variations within a given group were very great in many instances. The greatest regular variation was exhibited by the cereals and their products, in which group the maximum concentration (wheat bran) was 186 times as great as the minimum (corn flakes). Non-leafy vegetables were most uniform, with only a three-fold variation.

From the point of view of practical nutrition, the question arises as to what can or does supply the greatest share of manganese in the diet. Although nuts contain the highest concentration of the element, the intake of these is so low as to make them of little importance as sources of manganese for man. On the other hand, since the cereals and their products form such a large portion of the human diet, and since they also have a high concentration of the element, they probably contribute most to the supply of manganese in the diet. If calculated on the basis of food materials served in typical American diets as given by Rose (26, 27), the cereals and their products contribute about 38 per cent of the total manganese in two representative family menus. On the same basis of calculation, fruits and vegetables furnish 32 and 14 per cent respectively.

Only when the specific function or functions of manganese in the animal body have been uncovered and the amount needed daily for proper nutrition ascertained, can it be said that a given diet is adequate or deficient in this element. In view of the fairly high concentration of the element in cereals and their products as well as in fruits and vegetables, it appears unlikely that ill effects from inadequate amounts of manganese will result so long as these classes of food materials contribute their present share to the total food intake.

Summary

The manganese content of 83 representative food materials is given. The concentrations range from 0.028 mg. per liter of milk to 49.9 mg. per kilo. of bran flakes. Thirty-six of the materials contained less than 1 mg., twenty-five from 1 to 5 mg., and only twenty-two more than 5 mg. per kilo. of fresh material.

Arranged in descending order with respect to their manganese content, 12 classes of foods, representing 138 food materials, appear as follows: nuts; cereals and their products; dried legume seeds; green leafy vege-

tables; dried fruits; roots, tubers, and stalks; fresh fruits; non-leafy vegetables; animal tissue; poultry and poultry products; dairy products; and fish and sea foods.

Marked variations in manganese content were found among the several members of a given group.

In typical American diets cereals and their products contribute the largest proportion of the manganese intake.

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Editorial Review*

PHENOMENA OF RETARDED GROWTH

THE phenomenon of growth has long aroused the interest of investigators. The ordered accretion of living material, from the fertilized egg to the adult form has been considered a constant, a fundamental point of departure, for the studies of the biologist and, latterly, of the biochemist. This increase in the number of cells, in the size of the cells and in the material produced by the cells and deposited between them in the tissues is not, under ordinary circumstances, an irregular and uncontrolled activity. It normally takes place in certain more or less fixed intervals of time and the development of the various parts of the organism bears a definite relationship each to the other as well as to the growth of the organism as a whole. Furthermore, there are limitations upon the final size attained which are imposed by the germ plasm and which emphasize the fact that the phenomenon is characteristic of the species concerned. Heredity is, therefore, a primary factor governing growth and all growth therefore is, in a sense, restricted. However, in the present discussion the term "retarded growth" implies a limitation of development far more stringent than that imposed by the inherited tendencies of the organism.

Another factor governing growth is environment. While light, temperature and osmotic pressure may be of considerable importance in affecting development, the nature of the food materials is of great significance in furthering the optimal growth of which the species concerned is capable. Investigation in the field of nutrition has sought to evaluate the food requirements of the organism from both the qualitative and the quantitative point of view, for growth, maintenance, reproduction and in various abnormal conditions. In the course of some of these studies it has been observed that the development of experimental animals can be changed markedly by adjustment of the diet. The most convenient index of altered rate of growth is, of course, some measurement which can be made on the live, intact animal. It is natural, therefore, that observations on accelerated or arrested development have usually been based on changes in body weight or body size. While such criteria have yielded information of great value it is of interest to determine, if possible, other concomitants of altered rate of growth, such as structural changes not detectable in the intact

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organism, chemical variations in the tissues and body fluids and accompanying functional changes. Furthermore, in consideration of the various pathways taken by the digestion products of the common food principles in the course of metabolism, there seems little reason to expect that retardation of growth by the various devices thus far employed should always influence the development of the different parts of the body or their interrelationship in the same manner. This point of view has been justified by some of the older as well as by the more recent investigations in this phase of the field of nutrition.

Methods of Retarding Growth: Retardation of growth as measured by cessation of normal increase in body weight can be produced by various types of dietary adjustment. Strict limitation of the quantity of food given to an experimental animal will inevitably result in restriction of growth in proportion to the requirement of energy of the animal at that time. The young, developing organism has definite demands for food fuel and one of the early devices used to inhibit increase in weight was the restriction of the energy intake. However, with the growing conviction that certain constituents of the ration are indispensable for normal functions of the organism, there developed the realization that the gross restriction of food intake probably results in a deficiency condition which is exceedingly complex. When an unduly small quantity of a given food is allowed, not only is the energy ingestion curtailed, but the intake of the requisite vitamins, mineral salts and protein is likewise decreased. To eliminate the possibility of an actual shortage of such dietary essentials, the percentage composition of the experimental "low-calorie" diet is best adjusted so that the absolute daily intake of the indispensable components remains adequate in spite of the fact that the energy consumption is reduced (Winters, Smith and Mendel, 1927).

Limitation of ingested water is a possible method of retarding the development of the young animal, providing the experiment is of long enough duration. This is an especially severe restriction not only because water is an absolute necessity for the functions of the organism, but also because the young animal is normally more hydrated than the adult. Information of considerable fundamental importance could be elicited from such a study if the situation were not complicated by other dietary deficiencies. It has been demonstrated (Kudo, 1921; Jackson and Smith, 1931) that by limiting the intake of water the body weight of young rats can be maintained at a stationary level for a considerable portion of the life span of the animal. Also, deprivation of water is accompanied by a definite decrease in food consumption. The results of an experiment of this type must, there-

fore, be interpreted in the light of a deficiency of energy, water and, unless the ration has been suitably adjusted, of other indispensable factors. In the study of Jackson and Smith (1931) a group of animals consuming only as much food as was eaten by the test rats but receiving water *ad libitum*, served as a satisfactory control upon the factor of inanition. Under these conditions, effects specifically due to water deprivation were demonstrated.

Another method which has been used to retard the growth of young animals consists of restricting the quantity of protein consumed (Osborne and Mendel, 1915, 1916; Winters, Smith and Mendel, 1927). An experimental animal deprived of protein soon exhibits a complete loss of appetite. By regulating the quantity of protein in the diet, the rate of growth of the organism can, within limits, be controlled. This device will produce an uncomplicated "total nitrogen" deficiency only when the protein employed yields upon hydrolysis a complete series of the recognized essential amino acids in adequate quantity. Proteins which fail to yield requisite quantities of these indispensable amino acids will not satisfy the demands of the animal, in spite of the fact that the total amount of nitrogen provided would ordinarily be adequate. The amino acids whose indispensability for maintenance and growth rests on reasonably satisfactory experimental evidence are lysine, cystine, tryptophane and histidine (Osborne and Mendel, 1914; Rose and Cox, 1924; Johns and Finks, 1920); less convincing proof has been brought forward to indicate that the cyclic amino acids phenylalanine, tyrosine and proline are essential to physiological well being. It appears, then, that an experimental ration lacking or containing a limiting amount of an essential amino acid can serve to retard growth owing to a very specific dietary deficiency. The proteins gliadin from wheat and hordein from barley, both yielding on hydrolysis proportions of lysine too small to permit growth, have been employed extensively as the nitrogenous component of rations designed to maintain constant body weight of experimental animals without allowing growth.

Restriction of growth has been produced by feeding diets from which the inorganic salts have been removed to a large extent (Winters, Smith and Mendel, 1927; Smith and Swanson, 1929). While there is a moderate demand throughout life for minerals salts, this requirement is markedly accentuated in the young, growing mammal where tissues, notably those of the skeletal system, are being built up. The fact that in experimental animals restricted to such rations, the bones provide a readily available reservoir of certain highly important inorganic salts, renders the task of retarding growth by limiting the mineral elements of the diet extremely difficult. The problem of preparing a ration which is to be adequate in

all other respects than in its content of mineral salts, lies not with the organic components relied upon to yield the energy but with certain of the vitamin supplements. These consist usually of relatively impure substances with a considerable content of inorganic material. It thus happens that while one dietary essential is being incorporated in the food, the factor designed to be eliminated is included as a contaminant. As a result, students of the effect of limitation of mineral salts must employ a combination of vitamin supplements specially selected for their small content of inorganic salts.

It appears that the restriction of any constituent known to be indispensable in the diet is followed by interference with growth in the young and by difficulty of maintenance in adult animals. The lack of vitamins in the ration results in the appearance of changes which, because of the regularity with which they occur under prescribed conditions, have been considered as characteristic of lack of these food factors. The differentiation between the vitamins rests to a large extent upon the specificity of the changes produced by the absence of these adjuvants in the diet. Growth is inhibited in the absence of any of the recognized vitamins although doubt may reasonably be expressed as to whether or not this regularly observed cessation of growth is a primary or a secondary effect of the deficiency. While the limitation of vitamins in the ration is a device which can be depended upon to retard somatic development, the condition thus produced is an exceedingly complex one; there always occur accompanying structural and functional disturbances not apparent when, for instance, growth is restricted by underfeeding with a qualitatively adequate ration. It therefore follows that the classical symptoms of severe vitamin deficiency can scarcely be considered as phenomena of restricted growth alone.

The normal activity of the living cell is conditioned not only by the nature and amount of food materials brought to it but also by the efficiency of removal of the waste products of metabolism. In single-celled organisms excretion can only with difficulty be disturbed. On the other hand, in the higher animals incompetence of the apparatus for removal of waste products frequently exerts an untoward effect upon the well being of the animal. Evidence bearing upon this point was obtained from our studies of the growth of young rats after unilateral nephrectomy (Smith and Jones, 1927). A ration containing an extremely high concentration of protein retarded the growth of these animals whereas development was normal on a diet containing an ordinary amount of protein. It was suggested that the accumulation of nitrogenous metabolites exerted a generalized depres-

sing effect upon the organism which was reflected in the lowered food consumption and hence, in the growth. The high level of urea in the blood of these animals contrasted with that in intact animals on the same ration, supports this view.

Resumption of Growth: One of the phenomenal features of retarded growth is the retention, on the part of the experimental animal, of the ability to resume increase in body weight when the nutritive conditions once more become favorable. The consensus of opinion of many of the older investigators of problems of growth and nutrition was that the ability of the organism to increase in mass in the typical manner depended upon the exercise of this function during the portion of the life span in which it ordinarily takes place. In other words, it was assumed that unless growth occurs at the appropriate age, the ability to grow is, to a greater or less extent, lost. Experiments of Aron (1911) in which young dogs failed to regain the normal weight when they were realimented after long periods of maintenance at a markedly subnormal weight by underfeeding, favored this view. Studies with rats (Aron, 1914) likewise maintained on rations poor in protein, and experiments with very young rats which were underfed (Jackson and Stewart, 1920) showed a similar tendency toward the inability to regain the normal weight. On the other hand, the extensive investigations of Osborne and Mendel (1912, 1914 and 1915) indicate that the ability of the albino rat to resume growth is retained throughout prolonged periods of retarded development under the conditions of their experiments.

These investigators employed various devices for restricting the growth of their animals. Inadequate concentration of complete protein, deficiency of the essential amino acid lysine, insufficiency of energy and limitation of the amount of vitamins apparently produced, in general, similar results as concerns the maintenance of body weight and the subsequent ability to grow. Neither the body weight at which the maintenance was begun nor the duration of the stunting period had an influence upon the latent power to grow. Repeated instances of restricted development extending well beyond the age at which growth normally ceases are recorded in these studies; when the ration was made adequate growth was resumed and in many cases a body weight normal for the age ultimately reached. For instance, a female rat weighing 52 grams at 35 days was permitted to grow at a subnormal rate so that at 303 days of age she weighed only 73 grams. Upon realimentation, this rat grew at an accelerated rate until she weighed 376 grams 180 days later. The ability to reproduce was not lost in these animals and the milk of the mother provided for normal growth of the progeny during the period of lactation, although she herself was prevented

from growing by the defective dietary regime. These young grew at the usual rate when provided with satisfactory food after weaning. Not only is the very ability to resume growth remarkable, but the fact that the normal body weight for age of these previously retarded animals is frequently attained in a shorter time than usual, is a phenomenon worthy of note.

The persistence of the ability to grow has been shown in another way by studies (Smith and Moise, 1924) in which the rate of regeneration of injured liver tissue was measured in rats subsisting upon a ration deficient in lysine, the body weight of these young animals being thus maintained at a constant level. It was observed that, after a standard degree of hepatic necrosis induced by chloroform, the repair of the liver proceeded as rapidly in these stunted rats as in the control animals consuming an adequate ration. Here is evidence of the conservation of available essential material for reconstruction of a damaged vital organ at the same time that general somatic growth is not possible. Furthermore, it indicates that the chemical processes involved in repair may not be the same as those of growth.

This phenomenon of resumption of growth may be looked upon as a defense mechanism. The restriction of development resulting from adverse environmental conditions is automatically compensated by subsequent growth at an accelerated rate. Indeed it has been shown that not even under ideal conditions of nutrition does growth take place with complete uniformity. There are cycles of acceleration followed by periods of retardation in normal growth curves of animals and plants. These irregularities have been studied in man, fowl, rat, guinea pig, cow and mouse, as well as in plants and unicellular organisms (Robertson, 1923; Brody, 1928). The studies upon laboratory animals have indicated the surprising extent to which growth may be depressed without exceeding the limit of possibility of resumption of growth after favorable conditions are again established.

Change in Body Form: The change in conformation of the body is one of the early recognized and very constant accompaniments of maintenance of body weight at a subnormal level. One of the first series of systematic observations of this phenomenon was made by Waters (1908, 1909). In his studies a large number of young steers was underfed for varying periods up to a year in such a way that there was no increase in body weight. The form of the stunted animals was distinctly different from that of the control group; the length of the fore leg had increased and hence, the height at the withers, the length of the body was greater than at the beginning of the experiment, and the head became longer. There was also a dorsoventral elongation of the thorax. These changes took place at the expense

of adipose and muscular tissue and at a rate only moderately below normal. The resulting animals were emaciated, and obviously too tall and too long for their body weight. Similar observations were made by Aron (1911) upon puppies held at nearly constant weight by the limitation of their energy intake. In this case there was almost a normal increase in both length and height with a resulting distortion of bodily contour. It is unfortunate that in neither of the above-cited series of experiments were the stunted animals compared to normals of the same body weight, a comparison which brings out the alterations in body form much more clearly.

Somewhat similar changes in form have been reported in rats maintained at subnormal weights by limitation of water. Marked increase in length of tail and in weight of skeleton were observed under these conditions by Kudo (1921) though body length was little affected. These studies were of relatively short duration and it is uncertain whether water was the only deficiency in the diet. In experiments somewhat better controlled but with too few animals, Jackson and Smith (1930) showed an increase in body length:body weight ratio in rats maintained at a markedly subnormal weight by limitation of water intake. That a deficiency in vitamins results in a generally similar change in shape of the body has been shown by Quinn, King, and Dimit (1929). Quantities of vitamin A so small as to permit growth of only 22 to 30 grams in 8 weeks were given to very young rats with the result that at the end of this time there was evidence that the body length, leg length, chest girth and width of hips had increased to a relatively greater extent than had the body weight. Similar distortions of body form were detected in rats maintained at a constant weight through limitation of vitamin B (complex) by these investigators.

An attempt to differentiate between the structural effects of inhibition of growth by various methods was made by Winters, Smith, and Mendel (1927). Male rats weighing 40 to 50 grams were maintained for 40 days at that weight by giving one of the following deficient rations: low-calorie, low-protein, low-lysine and low-salt. Except for the deficiency indicated, the diets were adequate. At the end of the experimental period (40 days) it was found that, compared to normally growing rats of the same weight, the animals on each of the above diets had increased in body length although the weight had remained constant. This elongation was greatest in the group given the low-lysine food (8 per cent) and least with the animals receiving the diet poor in salts (3 per cent) although in every case the difference in length between the normal control and the stunted rats was statistically significant. It was also shown that the tails of the stunted rats were too long, most pronounced in the case of the low-salt animals.

The humeri, ulnae, radii, femora and tibiae were measured; these bones were 10 to 20 per cent longer than those of normal rats of the same weight. Comparing the expected growth with that obtained, it was found that during retarded development under these conditions, the stunted animals made from 9 to 20 per cent of the expected gain in body length and 36 to 40 per cent in length of leg bones. All groups of the stunted rats in these studies showed a slightly greater than normal size of cranium and length of mandible.

There is little room for doubt, on the basis of the available literature, that, whereas it is possible to retard the characteristic increase in body weight by a variety of methods involving an adjustment of the ration, little can be done to prevent the persistent growth of the bones. Thus, in spite of a stationary body weight, the physique of the experimental animal slowly changes, the length and height of the body increasing and the thoracic cavity becoming deeper and narrower. These alterations of body form are usually accentuated by the shift of material from the adipose and muscular tissue. It appears, furthermore, that under closely comparable experimental conditions the degree of structural change resulting from this persistent impulse to grow is dependent, to some extent, upon the nature of the deficiency in the ration.

Weight and Composition of Bones: Since growth is looked upon as a correlated increase of parts of the body, it might be expected that restriction of development of the organism as a whole would result in a proportional retardation of all the constituent parts. However, the facts already noted with reference to the linear increase of the long bones throw doubt upon the validity of such an assumption. One of the constant accompaniments of retarded growth is the increase in fresh weight of the bones. This was brought out in a striking manner by the study of Aron (1911) on two dogs from the same litter, one of which was permitted to grow and the other prevented from increasing in weight. At the end of 200 days the first animal weighed 5.8 kilos while the second weighed 2.85 kilos. In spite of this great discrepancy in body weight, the fresh weight of the bones of the stunted animal was only 6 per cent less than that of the well-fed dog. Restriction of water intake to the extent of retarding growth results in a definite increase in the weight of the bones (Kudo, 1921; Jackson and Smith, 1931). The various types of stunting employed by Winters, Smith, and Mendel (1927) resulted in increases in the dry weight of the bones up to 90 per cent of that of control animals of the same weight, the greatest accretion of weight taking place in the group of rats retarded by a deficient intake of energy ("low-calorie") and the least in the group on a diet poor

in mineral salts. All of the long bones in the leg were used in these determinations and, except in the "low-salt" group, each bone showed an increase in weight.

It thus becomes apparent that in the elongation of the bones brought about by the restriction of growth, there occurs concomitantly an increase in the weight of the bones, *i.e.*, the proportions of the individual bones remain more or less normal. Quinn, King, and Dimit (1929) found that with the possible exception of the humerus, rats whose growth was retarded through a lack of vitamin A had leg bones which, though too long for the body weight, were of normal proportions. In their group of animals upon a limited vitamin B intake the proportions of the humerus were abnormal though all the other leg bones measured showed the expected relationships between length and thickness. It therefore follows that the persistent increase in the weight of the bones, often in the normal proportions if not to the normal degree, takes place at the expense of other tissue constituents, the gross body weight being stationary, and that changes in composition of the bones might be expected under conditions of retarded growth.

A marked change in composition of the bones of his underfed dogs was observed by Aron (1911). In one experiment the bones of the stunted animal had approximately the same length and weight as those of a dog weighing twice as much. Upon analysis, however, it was found that the bones of the restricted animal had 17 per cent more water than those of the normal dog and that the fat had entirely disappeared, water in the starved bone taking the place of fat in the normal structure. On the other hand, there was only a slight reduction in the percentage of protein, and the ash content remained unchanged. These observations on simple underfeeding are of interest in the light of those (Smith and Schultz, 1930) with young rats maintained at constant body weight by strict limitation of inorganic salts. These animals were compared to normal rats of the same weight and to others of the same age. The fresh weight of the femora was greater than that of normal rats of the same body weight but less than that of controls of the same age. Growth of the bones had persisted in spite of the pronounced lack of the characteristic building material for this structure. The water content of the "low-salt" bones was much greater (16 per cent) than that of animals of the same age but somewhat less than that of the weight controls. The relative amounts of ash and fat in the bones of the stunted rats were the same as those of rats of the same body weight but when the alcohol-ether-insoluble organic residue of the "low-salt" bone was compared with the weight controls a marked difference in favor of the "low-salt" bones was apparent; indeed, the concentration was slightly greater

than that of normal rats which had grown to more than three times the body weight of the "low-salt" animals. The normal growth of bone in the rat is characterized by an increase in the percentage of organic residue (Hammett, 1925); the striking increase of this constituent in the rats upon the salt-poor ration affords still further confirmation of the persistence of the growth impulse in bone of animals in which increase of body weight is prevented. These studies, to be sure, represent a more specialized condition than in the case of gross underfeeding for here one of the specific bone constituents is lacking, in large measure, from the diet. The increased deposition of organic residue, the prepared pattern for real bone, offers a possible explanation for the accelerated development observed in these animals, once the ration is made adequate.[1]

Changes in Organs: Is it likely that other organs show, like the bone, a disproportionate change in size under conditions of restricted growth? It is especially reasonable to expect such changes because of the essential localization in certain organs of the metabolism of given food principles concerned in the stunting. Just as the deposition of salts in the bone is adversely affected by the consumption of rations extremely poor in salts, so the liver and kidneys may be influenced by adjustment of the dietary protein because these organs are especially involved in the deaminization of amino acids, the disposal of the non-nitrogenous rest, the synthesis of urea and finally, the excretion of the nitrogenous products of metabolism.

With the exception of the brain, the organs of dogs, maintained at a markedly subnormal weight by underfeeding, retained their weights and thus their normal relation to the body weight (Aron, 1911). The brain of the stunted animals continued to grow so that at the end of the experimental period, it constituted 2 per cent of the body weight whereas in the normal animal it was 0.9 per cent. Hence it appears that the internal organs are called upon less than other tissues to contribute substance to make good the continued growth of the skeleton. The data also show that there was a complete loss of stored body fat and a very large diminution of fat in the organs. A marked loss of total body protein occurred; this arose primarily from the muscles. On the other hand, there was found an increase in protein in the bones, a means whereby some of the muscle protein was conserved to the body. The loss in muscle protein and body and marrow fat is made up by an increased proportion of water. This explains one of the characteristic observations in the study of inanition, namely, that in animals subjected to dietary management which does not permit

[1] Unpublished observation from this laboratory by Mr. R. O. Brooke.

growth, the calorie value per gram of tissue is definitely lower than that of normally fed individuals.

That the changes in the size of organs are not the same in all types of restriction of growth by diet was shown by Winter, Smith, and Mendel (1927). In rats whose body weight was maintained at 40 to 50 grams for 40 days by insufficient energy intake, uncomplicated with other deficiencies, the liver, heart and kidney had a normal weight for animals of that size whereas the testes were markedly enlarged. When the rats were stunted by a deficiency of protein, the kidney and testes were normal but the liver and heart were enlarged. When the proportion of lysine in the ration was unsuited for growth, the liver had a normal size for body weight but the heart, kidney and testes were much too large. Strict limitation of inorganic salts was followed by a persistent growth of liver, heart, testes and kidney, the enlargement of the kidney under these conditions being especially striking. It was subsequently shown (Smith and Schultz, 1930) that this increase in weight of the kidney is accompanied by an augmented content of ash in the organ; and by the usual decrease in moisture with increasing age. Smith and Swanson (1931) presented supplementary data on the kidneys of rats given the salt-poor ration for 90 days, the body weight being maintained at 150 grams. After 21 days on the experimental ration, the same renal enlargement was observed as in the previous studies and the water content was normal. The age of the rat at this time was the same as that of the animals studied by Smith and Schultz. After 42, 63 and 90 days on the experimental diet there was a slight regression in weight but an increase in moisture content so that after 90 days the kidney, still too large for the body weight, was definitely hydrated. These studies were carried out upon comparable groups of animals and under carefully controlled dietary conditions in the light of modern nutritional principles. Large enough numbers were used so that the differences noted are statistically reliable.

A still different combination of effects upon the organ weights is brought about by a restriction of water (Jackson and Smith, 1931). In these experiments the eyeballs, kidneys, ovaries, testes, hypophysis and suprarenals were enlarged; the brain, stomach and muscle were unchanged and the liver, spleen, skin, thymus and thyroid decreased. Accompanying the changes in size there was a dehydration, the skin losing most water and the brain least. It appears that, although growth can be retarded and body weight maintained for relatively long periods by various deficient rations, a closer study of the detailed structural response of the individual organs

of the body indicates differences which are more or less characteristic of the dietary adjustment causing them.

Effects upon the Blood: Jackson (1925) has emphasized the constancy of the composition of the blood during inanition. Apparently there are few changes in this body fluid or its components which are characteristic of inanition and restricted growth. In a study of the blood of albino rats whose growth was retarded by strict limitation of inorganic salts during the period of rapid development, Smith and Swanson (1929) demonstrated a unique type of polycythemia. The erythrocytes were subnormal in size and the cell volume of the blood was therefore low. There was a marked reduction in corpuscular hemoglobin which, in spite of the large number of cells, resulted in anemia. However, the blood volume was normal and the concentration not increased. This condition was shown to be due to lack of salts rather than to the voluntarily reduced energy intake of the experimental animals. The marked changes in the blood did not appear unless there was a definite cessation of growth. When a complete salt mixture was given, the high erythrocyte count returned to normal, the hemoglobin increased, the cell volume increased and the previously high reticulocyte count showed a tendency to decrease (Swanson, Schultz and Smith, 1930). These phenomena are thus distinctly correlated with the sole dietary deficiency involved—namely, the lack of inorganic salts. Apparently there is a close dependence of both body weight and of normal blood composition upon a certain minimal concentration of mineral salts in the ration.

Influence on Behavior: In addition to the structural changes thus far discussed, it is reasonable to expect that such striking alterations in size, proportion and composition of organs would be accompanied by demonstrable changes in function. The question of the behavior of experimental animals which have been severely stunted during the period of most rapid growth is one of considerable significance. Will such individuals react to situations in the same manner as normal animals of the same size and, therefore, much younger, or can certain of the elements in the behavior be attributed to processes whose full development depends on age? Again, is it possible to establish a correlation between behavior and a certain type of dietary deficiency to the extent that such a correlation has been demonstrated between dietary deficiency and structural changes? In general, as the result of uncontrolled observation, it has been concluded that a malnourished child is likely to be dull and lethargic. Lusk (1921) has described the mental apathy, muscular inertia and difficulty of sustained effort among the population in Germany during the World War. On the other hand, there is some evidence pointing to the fact that undernourished

school children are not mentally inferior to normal ones and may be somewhat superior (Hunt, Johnson, and Lincoln, 1921).

The behavior of stunted animals has been compared to that of normal controls by Anderson and Smith (1926). This preliminary investigation indicated that rats stunted by underfeeding and by the lack of lysine were superior to normal animals of the same age in relearning an old problem as well as in learning a new one. The measure of performance was the speed and accuracy shown in running a moderately difficult maze. In order to obtain more varied measures of performance as well as to control the effect of age in the experimental groups, a second and more extensive series of studies was carried out (Anderson and Smith, 1931).

A large number of male rats 24 days old were divided into 10 groups, each with the same mean body weight and dispersion. One group was tested immediately and served as the normal weight control for the stunted animals. Forty days later, a group which had grown normally from the beginning, a group which had been given only enough of an adequate diet to maintain body weight at 40 grams and a group stunted with the gliadin ration were given the behavior trials; at 80 days after the beginning of the experiment similar groups appropriately fed from the beginning were tested and finally at 120 days three more groups, similarly fed, were tested. The measures of performance used were the speed and accuracy of learning to run through a moderately difficult maze, voluntary activity in a revolving cage, the time required to traverse a simple habituation box without culs-de-sac, and the time required to open the exit door of a problem box. The experimental rations were so constituted that, so far as could be gauged by present standards of nutrition, there was only the single deficiency in each case.

In practically all of the devices employed to measure the behavior of the animals there is a difference between the controls and the restricted animals and, in some instances, between the two types of stunting. As measured by the results in the activity cage, the low-calorie rats are the most active group at every age tested, the low-lysine, less active and the normal controls least active. With advancing age, the activity displayed by a given nutritional group decreases. In the stunted groups, therefore, one sees the persistence of the activity of youth in the general level of performance, upon which is superimposed the age factor of which the progressive decrease in activity is the evidence. Increasing the length of the stunting period prior to testing does not increase the activity of the rats in either of the restricted groups. One of the striking observations made was that after realimenting and after the body weight of the previously stunted

animals had almost reached that of the control rats, all differences in activity between the groups vanished. In this measure of simple activity there is a difference between the effects of the two types of stunting employed, the gliadin-fed rats behaving at all ages measured, very nearly like normal rats of the same body weight.

Rats receiving an insufficient energy allowance for growth run a simple habituation box and a fairly complex maze in less time than either normal animals of the same age or those similarly restricted through a deficiency of lysine. Here again, in those measures of performance involving speed, there tends to be a slowing-up with advancing age in all groups. On the other hand, it was found that the low-calorie group made the greatest number of errors while running the maze, the gliadin-fed rats somewhat fewer and the normal controls the least number. In other words, the stunted rats are extremely active but not accurate while the normal animals present the reverse behavior characteristics. Here again, as in the activity experiment, there is a differentiation on the basis of maze errors between the two restricted groups. Both the normal rats and those of the gliadin-fed groups become somewhat more accurate with advancing age; this is not true for the low-calorie groups. Strikingly enough, after the erratic, stunted rats have been realimented they and the normal controls exhibit a higher degree of accuracy than before but the previously stunted rats still make more errors than the controls, a fact suggesting that the error-making habit characteristic of their earlier trials has persisted in spite of the change in diet.

In solving the problem box situation the rats lacking lysine tend to be superior. In all groups the older rats are able to make a better record with this measure of performance than the younger ones. After realimentation all three groups solve the problem box better than they did before but the low-calorie group exhibits a persistence of inferiority. It appears, in general, that on the basis of age within groups, youth favors those measures depending upon speed while greater accuracy is shown by the older animals.

One of the uncertainties concerning the experimental animals whose growth has been retarded lies in the propriety of considering them as typical of normal individuals of the same size versus those of the same age. A very young rat maintained upon a ratio poor in lysine at its weaning weight of 45 grams for 40 days during which interval of time its litter-mate consuming an adequate ration has more than tripled its weight, resembles a normal animal of the size in weight only. The above discussion has pointed out the striking feature of continued growth of the skeletal system in all

types of retarded development thus far studied. Furthermore, certain of the organs change, not uniformly under all experimental conditions, to be sure, but regularly enough both in size and in composition to emphasize the fact that, in spite of a body weight maintained at a youthful or subnormal level, the animal is not like a young rat either from the structural or from the chemical point of view. A stunted rat bears little resemblance to the normal young individual of the same weight; the head and nose are somewhat longer, the body is elongated, the legs are longer and there is a typical gaunt appearance which becomes more pronounced the longer the duration of inhibited growth. On the other hand, the restricted animal is not like a normal rat of the same age either in proportion or in composition.

On the basis of the results of the behavior studies it is equally impossible to classify a stunted individual exclusively either with the youthful group or with the mature age controls. In general, in measures of behavior involving speed, the restricted animals tend to resemble the normals of the same body weight while in the problem box they approach more closely the age controls. In no sense can it be said that an animal whose growth has been severely retarded has retained entirely its youthful characteristics or has attained completely those typical of its age. Such an individual is essentially a different organism with a structure, composition, behavior and, possibly, a metabolism, more or less characteristic of this state of existence.

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